

**APPLICATION FOR UNITED STATES LETTERS PATENT**

**ENTITLED**

**DEFECTS IN PERIAXIN ASSOCIATED WITH  
MYELINOPATHIES**

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Utility Application

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# DEFECTS IN PERIAXIN ASSOCIATED WITH MYELINOPATHIES

[0001] The present invention was developed with funds from the United States Government. Therefore, the United States Government may have certain rights in the invention.

[0002] The present invention claims priority to U.S. Provisional Patent Application 60/255,217, filed December 13, 2000.

## FIELD OF THE INVENTION

[0003] The present invention is directed to the fields of molecular biology, molecular genetics, and neurology. More specifically, the present invention is directed to defects in periaxin related to neuropathies. More specifically, the neuropathies include recessive Dejerine-Sottas and Charcot-Marie-Tooth disease.

## BACKGROUND OF THE INVENTION

[0004] Dejerine-Sottas neuropathy (DSN) and Charcot-Marie-Tooth disease type 1 (CMT1) represent genetically heterogeneous inherited peripheral myelinopathies. These conditions constitute part of a spectrum of neuropathy phenotypes ranging in severity from congenital hypomyelinating neuropathy (CHN) to adult onset hereditary neuropathy with liability to pressure palsies (HNPP) (Lupski and Garcia, 2001). At least fifteen genetic loci and six genes have been associated with these disorders; identified genetic causes include altered dosage of peripheral myelin protein 22 (PMP22) or mutations in one of the following genes: *PMP22*, the gap junction protein  $\beta 1$  gene (*GJB1*), the myelin protein zero gene (*MPZ*), the early growth response gene 2 (*EGR2*), the myotubularin related protein 2 gene (*MTMR2*), or the *N-myc* downstream regulated gene 1 (*NDRG1*) (Lupski and Garcia, 2001). These genes encode proteins of diverse functions: compact myelin structural proteins (*MPZ*, *PMP22*), a non-compact myelin gap junction protein (*GJB1*), signal transduction proteins (*NDRG1*, *MTMR2*), and a transcription factor for late myelin genes (*EGR2*). Both dominant (*PMP22*, *GJB1*, *MPZ*, *EGR2*) and recessive (*MTMR2*, *NDRG1*, *PMP22*, *EGR2*) mutant alleles have been described. Historically considered an autosomal recessive disorder (Dejerine and Sottas, 1893), DSN has been associated predominately with *de novo* dominant mutations in *PMP22* (Roa *et al.*, 1993), *MPZ* (Hayasaka *et al.*, 1993), or *EGR2* (Timmerman

*et al.*, 1999), although rare recessive mutations in *PMP22* have also been reported (Lupski, 2000; Parman *et al.*, 1999).

**[0005]** In murine embryonic Schwann cells, L-periaxin is initially concentrated in the nuclei but redistributes to the plasma membrane, predominantly adaxonal, with initiation of myelination and then to the abaxonal, Schmidt-Lanterman incisures, and paranodal membranes with maturation of the myelin sheath (Scherer *et al.*, 1995; Sherman and Brophy, 2000). In addition, L-periaxin expression recapitulates this pattern following crush injury (Scherer *et al.*, 1995). This shift in periaxin localization after the spiralization phase of myelination suggests that periaxin participates in membrane-protein interactions that are required to stabilize the mature myelin sheath. As a cytoskeleton-associated protein, L-periaxin may mediate such stabilization by facilitating integration of extracellular signaling through the cytoskeleton which is essential for changes in Schwann cell shape and regulation of gene expression during axonal ensheathment (Fernandez-Valle *et al.*, 1997; Tapon and Hall, 1997). Such a signaling function is supported by the observation that L-periaxin contains a PDZ motif, a domain implicated in the assembly of signaling complexes at sites of cell-cell contact, and a nuclear localization signal (Dytrych *et al.*, 1998; Sherman and Brophy, 2000). Confirming the necessity of periaxin for maintenance of the myelin sheath, Gillespie *et al.* recently demonstrated that *Prx*<sup>-/-</sup> mice ensheath and myelinate peripheral axons apparently normally but subsequently develop a severe demyelinating neuropathy associated with allodynia (pain from non-noxious stimuli) and hyperalgesia (hypersensitivity to pain) (Gillespie *et al.*, 2000).

**[0006]** However, it was heretofore unknown in the art whether a relationship between the human *PRX* gene defects and neuropathies such as recessive DSN existed.

## SUMMARY OF THE INVENTION

**[0007]** In an embodiment of the present invention there is a method of diagnosing myelinopathy in an individual comprising the steps of obtaining a sample containing nucleic acid from the individual; assaying the sample for an alteration in a periaxin polynucleotide, wherein the alteration is associated with the myelinopathy. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy syndrome (RLS). In another specific embodiment, the assaying step further comprises a

polymerase chain reaction. In a further specific embodiment, the primers for said polymerase chain reaction are selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26. In a specific embodiment, the alteration is 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, or 2655T>C.

**[0008]** In another embodiment of the present invention there is a method of diagnosing myelinopathy in an individual comprising the steps of obtaining a sample containing protein from the individual; assaying the sample for an alteration in a periaxin polypeptide, wherein the alteration is associated with the myelinopathy. In a specific embodiment, the alteration is E1259K, A406T, E1359del $\Delta$ , E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, P885P, R953X, R368X, S929fsX957, R196X, V763fsX774, C715X, or R82fsX96. In another specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0009]** In another embodiment of the present invention there is as a composition of matter a defect of a periaxin polynucleotide of 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, and 2655T>C.

**[0010]** In an additional embodiment of the present invention there as a composition of matter a periaxin polypeptide defect of E1259K, A406T, E1359del $\Delta$ , E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, P885P, R953X, R368X, S929fsX957, R196X, V763fsX774, C715X, or R82fsX96.

**[0011]** In another embodiment of the present invention there is a method of identifying a compound for the treatment of myelinopathy comprising the steps of exposing the compound to a knockout animal, wherein the animal comprises at least one defective allele of a periaxin polynucleotide and wherein the animal has at least one symptom associated with the myelinopathy; and assaying for an improvement in said at least one symptom of the myelinopathy after exposure to the compound. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT)



syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0012]** In an additional embodiment of the present invention there is a method of screening for a compound for the treatment of myelinopathy comprising the steps of providing a cell lacking a functional periaxin amino acid sequence contacting the cell with the compound; and determining the effect of the compound on the cell, wherein said effect on the cell is indicative of the treatment of the myelinopathy. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0013]** In a further embodiment of the present invention there is a method of identifying an upregulator of periaxin nucleic acid sequence expression comprising the steps of administering a test compound to a transgenic animal, wherein the genome of the transgenic animal comprises a reporter nucleic acid sequence, wherein the sequence is under the control of an operably linked periaxin promoter active in eukaryotic cells; measuring the level of said periaxin expression; and comparing the level of the periaxin expression in the animal with normal periaxin expression, wherein an increase in the level following administration of the test compound indicates the test compound is an upregulator.

**[0014]** In another embodiment of the present invention there is a method of identifying a drug having activity in the treatment of myelinopathy, comprising the steps of obtaining a compound suspected of having extracellular signaling activity; and determining whether the compound has the extracellular signaling activity. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0015]** In another embodiment of the present invention, there is a method of treating myelinopathy in an organism, comprising the step of administering to the organism a therapeutically effective amount of a periaxin nucleic acid sequence, wherein the nucleic acid sequence is administered by a vector. In a specific embodiment, the vector is selected from

the group consisting of a plasmid, a viral vector, a lipid, a liposome, a polypeptide, or a combination thereof. In another specific embodiment the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0016]** In another embodiment of the present invention there is a method of treating myelinopathy in an organism comprising the step of administering to said organism a therapeutically effective amount of a periaxin amino acid sequence, wherein said amino acid sequence is administered with a physiologically acceptable carrier. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0017]** In an additional embodiment of the present invention there is a method of treating an animal for a myelinopathy comprising the steps of identifying a compound which interacts with a periaxin polypeptide; and administering to the animal a therapeutically effective amount of the compound. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0018]** In another embodiment of the present invention there is a method of treating a patient for a myelinopathy comprising the steps of preparing a compound obtained by a method from herein and administering the compound with a physiologically acceptable carrier to said patient.

**[0019]** In another embodiment of the present invention there is a kit for diagnosing a myelinopathy in an animal comprising at least two primers, wherein one primer is specific to a sense periaxin nucleic acid sequence and another primer is specific to an antisense periaxin nucleic acid sequence. In a specific embodiment the primers are SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:26.

[0020] In another embodiment of the present invention there is as a composition of matter a nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:26.

[0021] In an additional embodiment of the present invention, there is a method of detecting the presence or absence of a mutation associated with a myelinopathy, the method comprising a) isolating a test nucleic acid from a subject, said test nucleic acid comprising a periaxin polynucleotide; b) comparing the test nucleic acid to a reference wild-type periaxin polynucleotide; and c) determining the differences between the test nucleic acid and the reference wild-type periaxin polynucleotide, wherein the differences are mutations in the periaxin polynucleotide of the subject, and wherein the presence of a mutation in the periaxin polynucleotide of the subject is indicative of the presence of the myelinopathy in the subject.

[0022] In a specific embodiment, the mutation is in SEQ ID NO:1 and is 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, 2655T>C, 2145T>A, or 247ΔC. In another specific embodiment, the mutation encodes a defect of an amino acid sequence of SEQ ID NO:2 and is E1259K, A406T, E1359delΔ, E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, P885P, R953X, R368X, S929fsX957, R196X, V763fsX774, C715X, or R82fsX96. In another specific embodiment, the periaxin polynucleotide is SEQ ID NO:1. In an additional specific embodiment, the comparing step is by DHPLC, sequencing, or hybridization.

[0023] Other and further objects, features, and advantages would be apparent and eventually more readily understood by reading the following specification and by reference to the accompanying drawings forming a part thereof, or any examples of the presently preferred embodiments of the invention are given for the purpose of the disclosure.

#### BRIEF DESCRIPTION OF THE FIGURES

[0024] FIGS. 1a through 1c illustrate mapping of *PRX* and expression of *PRX* mRNA.

[0025] FIG. 1a demonstrates that by ePCR and fluorescent in situ hybridization (FISH), BAC CTC-492K19, which contains *PRX*, maps between *D19S324* and *D19S223*.

[0026] FIG. 1b illustrates a diagram showing the two *PRX* mRNAs resulting from alternative retention of intron 6. The large periaxin protein (L-PRX) is encoded by the shorter spliced mRNA and the smaller periaxin protein (S-PRX) by the longer mRNA retaining intron 6. Coding regions are shaded.

[0027] FIG. 1c demonstrates northern blot analysis of both the 5.1 and 5.6 kb *PRX* mRNAs.

[0028] FIGS. 2A through 2B demonstrate a comparison of human, murine and rat L-periaxin (2A) and S-periaxin (2B) amino acid sequences.

[0029] FIG. 2A shows human L-periaxin having approximately 78 and 73 percent sequence identity with the murine and rat proteins, respectively. The PDZ domain, tripartite nuclear localization signal (NLS1, NLS2, NLS3), repeat domain and acidic domain previously characterized in mice and rats are conserved in humans. Arrowheads indicate mutations identified in patients.

[0030] FIG. 2b shows S- and L-periaxin share a common amino terminal, but retention of intron 6 in the mRNA encoding S-periaxin results in a truncated protein with 20 amino acids encoded within the intron (black box). Identical amino acids are indicated by a colon (:), gaps by a dash (-) and stop codons by an asterisk (\*).

[0031] FIG. 3 illustrates chromatograms of *PRX* alterations identified in three families.

[0032] FIG. 4 demonstrates mutations identified in *PRX*. The location of mutations within L-periaxin is indicated in the diagram at the top by the arrows. The clinical phenotype of each patient, their mutations, and the frequency of their mutations in North American control chromosomes are listed in the table on the bottom of the figure.

[0033] FIG. 5 shows chromatograms of *PRX* alterations identified in two families. Standard pedigree symbols are used; males are represented by squares, females by circles. Black filled symbols indicate patients with CMT or CHN. Families PN-44, and PN-761 exhibit autosomal recessive inheritance. Below each individual the DNA sequence chromatogram is shown with the specific mutation (vertical arrows) given under the chromatogram. PN-44.1 and PN-44.4 are homozygous and their unaffected sister PN-44.3 is heterozygous for the C715X mutation. PN-761.3 is homozygous, and her parents and unaffected brother are heterozygous for the R82fsX96 mutation.

**[0034]** FIG. 6 demonstrates light microscopy of semi-thin resin sections from the sural nerve biopsies of patients PN-44.1 (6A) and PN-761.3 (6B). In FIG. 6A, there is a transverse section showing loss of myelinated fibers of all sizes, onion bulbs (arrows) which were sometimes denervated (arrowheads) and tomacula formations. A complex of grouped, proliferated Schwann cell processes surrounded a myelinated axon (inset). (Magnification x 637). In FIG. 6B, there is increased connective tissue and demyelinated (thin arrows) or thinly remyelinated (arrowheads) nerve fibers. Atrophic axons with relatively thick myelin sheaths are also apparent. A tomaculous fiber is indicated by a thick arrow, a demyelinated axon by a thin arrow, and thinly remyelinated fibers by arrowheads. (Magnification x 900.)

**[0035]** FIG. 7 shows electron microscopy of longitudinal sections in patients PN-761.3 (7A) and PN-44.1 (7B), and control sural nerve (7C). In FIG. 7A, there is heminode showing paranodal myelin folds and a Schwann cell process separating terminal myelin loops from the axon. (Magnification x 11,600.) In FIG. 7B, there are abnormalities of paranodal myelin loops and the absence of septate-like junctions or transverse bands (arrows). The myelin loops and axon are separated by a Schwann cell process (\*). There were no abnormalities of the myelin packing. (Scale 0.1  $\mu$ m). In FIG. 7C, normal myelin is shown. Note the well-developed septate-like junctions or transverse bands (arrows) of normal paranodal myelin. Also note the desmosome-like structures (big arrow). (Scale 0.1  $\mu$ m.)

**[0036]** FIG. 8 demonstrates immunofluorescence analysis of sural nerve biopsy from patient PN-44.1. For FIGS. 8A, 8B, and 8C, nerve fibers from patient PN-44.1 are shown staining with N-terminal PRX antibody (8A), and MBP (8B), which colocalise (8C). In FIGS. 8D, 8E, and 8F, nerve fibers are shown from patient PN-44.1 show staining with repeat region PRX antibody (8D), and MBP (8E), which colocalise (8F). In FIGS. 8G, 8H, and 8I, there are nerve fibers from patient PN-44.1 show no staining with C-terminal PRX antibody (8G), but staining with MBP (8H), indicating that a truncated PRX protein is formed. In FIGS. 8J, 8K, and 8L, there are nerve fibers from a normal control show staining with the C-terminal PRX antibody (8J), and MBP (8K), which colocalise (8L). Note the organized staining of PRX in the Schmidt-Lantermann incisures, which is not present in the patient. (Scale 5  $\mu$ m)

**[0037]** FIG. 9 provides a summary of all reported mutations identified in *PRX*. The top panel depicts which exons encode specific portion of the periaxin proteins. The middle panel of horizontal shaded boxes illustrates the various domains of L- and S-periaxin.

Previously reported mutations are given below with the mutations reported herein shown above. The location of mutations is indicated by the vertical arrows. The mutation C715X affects only L-periaxin, and the mutation R82fsX96 affects both S- and L-periaxin. The table at the bottom lists all reported PRX mutations and their associated disease phenotypes. Abbreviations; PDZ, PDZ domain; NLS, nuclear localization signal; DRP2, DRP2 binding domain.

## DESCRIPTION OF THE INVENTION

**[0038]** It is readily apparent to one skilled in the art that various embodiments and modifications may be made to the invention disclosed in this Application without departing from the scope and spirit of the invention.

**[0039]** As used in the specification, "a" or "an" may mean one or more. As used in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

### I. Definitions

**[0040]** The term "extracellular signaling activity" as used herein is defined as the function of mediating, facilitating integration with other factors of, or contributing either directly or indirectly to signaling between a factor outside the cell and a factor inside the cell, such as the cytoskeleton. In a preferred embodiment, the extracellular signaling through the cytoskeleton is essential for changes in Schwann cell shape and regulation of gene expression during axonal ensheathment.

**[0041]** The term "fragments or derivatives" as used herein is defined as portions or variants of a specific nucleic acid or amino acid which retains at least one specific function of the parent sequence. For instance, if an amino acid sequence of SEQ ID NO:1 is utilized for extracellular signaling, then the fragment or derivative would also have extracellular signaling activity. Alternatively, if an amino acid sequence (such as a peptide, polypeptide or protein) of SEQ ID NO:1 is utilized for interacting with another polypeptide or nucleic acid, such as in a complex, then the fragment or derivative of SEQ ID NO:1 would likewise interact with the polypeptide or nucleic acid. The fragments may be from any location within the nucleic acid or amino acid sequence and may be of any size up to the full sequence size. Derivatives may comprise a mutation, translocation, deletion, duplication, polymorphism, such as a single nucleotide polymorphism, insertion, and others known to a skilled artisan.

Derivatives of an amino acid sequence, such as a polypeptide, may contain at least one modification of at least one amino acid residue, such as methylation, phosphorylation, acetylation, or other modifications standard in the art.

**[0042]** The term “myelinopathy” as used herein is defined as a defect in myelin, a lipid substance which forms a sheath around nerve fibers. The defect may be absence of myelin, loss of myelin, or faulty myelin. In specific embodiments, the myelinopathy results in Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and/or Roussy-Levy Syndrome. A skilled artisan is aware myelin is also referred to as a white substance representing membrane extensions of Schwann cells which ensheath the peripheral nerve axon. Peripheral nerve myelinopathy refers to myelin of the peripheral nerve.

**[0043]** The terms “neuropathy” or “neuropathies” as used herein is defined as a functional defect or defects and/or a pathological change or changes in the peripheral nervous system. In a specific embodiment, the neuropathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and/or Roussy-Levy Syndrome.

**[0044]** The term “periaxin promoter” as used herein is defined as a nucleic acid sequence which under native conditions regulates expression of a periaxin nucleic acid sequence. The promoter may be from any organism.

**[0045]** The term “vector” is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated.

## **II. The Present Invention**

### **A. Embodiments**

**[0046]** In an embodiment of the present invention there is a method of diagnosing myelinopathy in an individual comprising the steps of obtaining a sample containing nucleic acid from the individual; assaying the sample for an alteration in a nucleic acid sequence of SEQ ID NO:1, wherein the alteration is associated with the myelinopathy. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy

syndrome (RLS). In another specific embodiment, the assaying step further comprises a polymerase chain reaction. In a further specific embodiment, the primers for said polymerase chain reaction are selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26. In a specific embodiment, the alteration is in SEQ ID NO:1 and is selected from the group consisting of 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, and 2655T>C.

**[0047]** In another embodiment of the present invention there is a method of diagnosing myelinopathy in an individual comprising the steps of obtaining a sample containing protein from the individual; assaying the sample for an alteration in an amino acid sequence of SEQ ID NO:2, wherein the alteration is associated with the myelinopathy. In a specific embodiment, the alteration is in SEQ ID NO:2 and is selected from the group consisting of E1259K, A406T, E1359Δ, E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, and P885P. In another specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0048]** In another embodiment of the present invention there is as a composition of matter a defect of a nucleic acid sequence of SEQ ID NO:1 and is selected from the group consisting of 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, and 2655T>C.

**[0049]** In an additional embodiment of the present invention there as a composition of matter a defect of an amino acid sequence of SEQ ID NO:2 and is selected from the group consisting of E1259K, A406T, E1359Δ, E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, and P885P.

**[0050]** In another embodiment of the present invention there is a method of identifying a compound for the treatment of myelinopathy comprising the steps of exposing the compound to a knockout animal, wherein the animal comprises at least one defective allele of a nucleic acid sequence of SEQ ID NO:1 and wherein the animal has at least one



symptom associated with the myelinopathy; and assaying for an improvement in said at least one symptom of the myelinopathy after exposure to the compound. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0051]** In an additional embodiment of the present invention there is a method of screening for a compound for the treatment of myelinopathy comprising the steps of providing a cell lacking a functional periaxin amino acid sequence contacting the cell with the compound; and determining the effect of the compound on the cell, wherein said effect on the cell is indicative of the treatment of the myelinopathy. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0052]** In a further embodiment of the present invention there is a method of identifying an upregulator of periaxin nucleic acid sequence expression comprising the steps of administering a test compound to a transgenic animal, wherein the genome of the transgenic animal comprises a reporter nucleic acid sequence, wherein the sequence is under the control of an operably linked periaxin promoter active in eukaryotic cells; measuring the level of said periaxin expression; and comparing the level of the periaxin expression in the animal with normal periaxin expression, wherein an increase in the level following administration of the test compound indicates the test compound is an upregulator.

**[0053]** In another embodiment of the present invention there is a method of identifying a drug having activity in the treatment of myelinopathy, comprising the steps of obtaining a compound suspected of having extracellular signaling activity; and determining whether the compound has the extracellular signaling activity. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0054]** In another embodiment of the present invention, there is a method of treating myelinopathy in an organism, comprising the step of administering to the organism a therapeutically effective amount of a periaxin nucleic acid sequence, wherein the nucleic acid sequence is administered by a vector. In a specific embodiment, the vector is selected from the group consisting of a plasmid, a viral vector, a lipid, a liposome, a polypeptide, or a combination thereof. In another specific embodiment the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0055]** In another embodiment of the present invention there is a method of treating myelinopathy in an organism comprising the step of administering to said organism a therapeutically effective amount of a periaxin amino acid sequence, wherein said amino acid sequence is administered with a physiologically acceptable carrier. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0056]** In an additional embodiment of the present invention there is a method of treating an animal for a myelinopathy comprising the steps of identifying a compound which interacts with an amino acid sequence of SEQ ID NO:2; and administering to the animal a therapeutically effective amount of the compound. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0057]** In another embodiment of the present invention there is a method of treating a patient for a myelinopathy comprising the steps of preparing a compound obtained by a method from herein and administering the compound with a physiologically acceptable carrier to said patient.

**[0058]** In another embodiment of the present invention there is a kit for diagnosing a myelinopathy in an animal comprising at least two primers, wherein one primer is specific to a sense periaxin nucleic acid sequence and another primer is specific to an antisense periaxin

nucleic acid sequence. In a specific embodiment the primers are selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

**[0059]** In another embodiment of the present invention there is as a composition of matter a nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, or SEQ ID NO:26.

**[0060]** In an additional embodiment of the present invention, there is a method of detecting the presence or absence of a mutation associated with a myelinopathy, the method comprising a) isolating a test nucleic acid from a subject, said test nucleic acid comprising a periaxin polynucleotide; b) comparing the test nucleic acid to a reference wild-type periaxin polynucleotide; and c) determining the differences between the test nucleic acid and the reference wild-type periaxin polynucleotide, wherein the differences are mutations in the periaxin polynucleotide of the subject, and wherein the presence of a mutation in the periaxin polynucleotide of the subject is indicative of the presence of the myelinopathy in the subject.

**[0061]** In a specific embodiment, the mutation is in SEQ ID NO:1 and is 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, 2655T>C, 2145T>A, or 247ΔC. In another specific embodiment, the mutation encodes a defect of an amino acid sequence of SEQ ID NO:2 and is E1259K, A406T, E1359Δ, E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, P885P, C715X, or R82fsX96. In another specific embodiment, the periaxin polynucleotide is SEQ ID NO:1. In an additional specific embodiment, the comparing step is by DHPLC, sequencing, or hybridization.

**[0062]** In a preferred embodiment, the human orthologue of murine and rat periaxin (*Prx*), which expresses L- and S-periaxin by alternative intron retention (Dytrych *et al.*, 1998), is associated with human inherited myelinopathies. The human periaxin gene (*PRX*) encodes two PDZ domain proteins, L- and S-periaxin, that are required for maintenance of

peripheral nerve myelin. *Prx*<sup>-/-</sup> mice develop a severe demyelinating peripheral neuropathy despite apparently normal initial formation of myelin sheaths. In preferred embodiments, mutations in *PRX* cause human peripheral myelinopathies given that multiple unrelated Dejerine-Sottas neuropathy (DSN) patients with recessive *PRX* mutations, two with compound heterozygous nonsense and frameshift mutations and one with a homozygous frameshift mutation were identified. The *PRX* locus was mapped to 19q13.13-13.2, a region recently associated with a severe autosomal recessive demyelinating neuropathy in a Lebanese family (Delague *et al.* 2000) and syntenic to the location of *Prx* on murine chromosome 7 (Gillespie *et al.* 1997).

**[0063]** The skilled artisan is made aware of the following GenBank accession numbers and URLs for data in this article are as follows: 1) Online Mendelian Inheritance in Man: (OMIM), <http://www3.ncbi.nlm.nih.gov/Omim/> (for CMT1 (MIM (Mendelian Inheritance in Man) #118200), DSN (OMIM #145900), CHN (OMIM #605253), and HNPP (OMIM #162500)); 2) GenBank, <http://www.ncbi.nlm.nih.gov/Genbank> (for human *PRX* mRNA sequence encoding S-periaxin (AF321192; SEQ ID NO:64) and human *PRX* mRNA sequence encoding L-periaxin (AF321191; SEQ ID NO:65)); 3) HUGO Gene Nomenclature Committee, <http://www.gene.ucl.ac.uk/nomenclature/>; 4) BLAST, <http://www.ncbi.nlm.nih.gov/BLAST/>; 5) Chromosome 19 physical map, [http://greengenes.llnl.gov/genome/html/chrom\\_map.html](http://greengenes.llnl.gov/genome/html/chrom_map.html); 6) Electronic PCR, <http://www.ncbi.nlm.nih.gov/genome/sts/epcr.cgi>; 7) Primer v3 program, [http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

**[0064]** A skilled artisan is aware that various periaxin sequences are within the scope of the compositions and methods of the present invention. Periaxin nucleic acid sequences within the scope of the present invention include: SEQ ID NO:1, BF447393 (SEQ ID NO:27); BF445684 (SEQ ID NO:28); NM\_019412. (SEQ ID NO:29); BE845046 (SEQ ID NO:30); BE627424 (SEQ ID NO:31); BE625883 (SEQ ID NO:32); BE504988 (SEQ ID NO:33); BB293550 (SEQ ID NO:34); BB197351 (SEQ ID NO:35); BB095645 (SEQ ID NO:36); BB095557 (SEQ ID NO:37); BG142832 (SEQ ID NO:38); AW590908 (SEQ ID NO:39); AW337783 (SEQ ID NO:40); AW212122 (SEQ ID NO:41); AW211564 (SEQ ID NO:42); AW180312 (SEQ ID NO:43); AV313851 (SEQ ID NO:44); AV232603 (SEQ ID NO:45); AW134382 (SEQ ID NO:46); AW105547 (SEQ ID NO:47); AI447899 (SEQ ID NO:48); AI637869 (SEQ ID NO:49); AI561629 (SEQ ID NO:50); AI551992 (SEQ ID

NO:51); AI466086 (SEQ ID NO:52); AI159496 (SEQ ID NO:53); AI159096 (SEQ ID NO:54); AA989929 (SEQ ID NO:55); AA984421 (SEQ ID NO:56); AJ222969 (SEQ ID NO:57); AJ222968 (SEQ ID NO:58); AA823031 (SEQ ID NO:59); AA727568 (SEQ ID NO:60); AA145455 (SEQ ID NO:61); AA105722 (SEQ ID NO:62); Z29649 (SEQ ID NO:63); BF476730 (SEQ ID NO:64); BG141436 (SEQ ID NO:65); BF940815 (SEQ ID NO:66); BF589760 (SEQ ID NO:67); XM\_068146 (SEQ ID NO:68); XM\_043307 (SEQ ID NO:69); XM\_047407 (SEQ ID NO:70); XM\_015939 (SEQ ID NO:71); XM\_047408 (SEQ ID NO:72); AY054648 (SEQ ID NO:73); BI315105 (SEQ ID NO:74); AF321192 (SEQ ID NO:75); AF321191 (SEQ ID NO:76); and/or NM\_023976 (SEQ ID NO:77). A skilled artisan recognizes how to find, based on common known methods in the art, mutations of a periaxin polynucleotide listed herein in another periaxin polynucleotide sequence even if that sequence comprises a larger or smaller region of the gene.

**[0065]** Periaxin amino acid sequences within the scope of the present invention include: SEQ ID NO:2 (NP\_066007); T49945 (SEQ ID NO:78); I58157 (SEQ ID NO:79); NP\_062285 (SEQ ID NO:80); Q10018 (SEQ ID NO:81); Q63425 (SEQ ID NO:82); CAB89377 (SEQ ID NO:83); CAA11023 (SEQ ID NO:84); CAA11022 (SEQ ID NO:85); CAA82757 (SEQ ID NO:86); NP\_076466 (SEQ ID NO:87); AAK19279 (SEQ ID NO:88); AAK19280 (SEQ ID NO:89); NP\_196515 (SEQ ID NO:90); AAK96839 (SEQ ID NO:91); XP\_047408 (SEQ ID NO:92); XP\_068146 (SEQ ID NO:93).

**[0066]** In a specific embodiment of the present invention there is a transgenic non-human animal, wherein although the animal is not a human animal, such as a mouse, the genetic material which comprises the transgene or any related sequences to the may be derived from a human. For example, a human periaxin nucleic acid sequence, such as SEQ ID NO:1, or a fragment thereof, may be introduced into a mouse or rat.

**[0067]** In a specific embodiment there is a method of identifying an upregulator of *PRX* expression comprising the steps of administering a test compound to a transgenic non-human animal comprising a nucleic acid encoding SEQ ID NO:1, wherein said nucleic acid is under the control of a promoter active in eukaryotic cells, and wherein said nucleic acid is endogenous to an animal other than said transgenic non-human animal; measuring the level of *PRX* expression; and comparing the level of *PRX* expression in said animal with normal *PRX* expression, wherein an increase in said level following administration of said test compound indicates said test compound is an upregulator. The term "normal *PRX*

expression” as used herein is defined as the basal level of expression of a PRX nucleic acid sequence. That is, the level of normal *PRX* expression is approximately the amount present in tissues in which it is expressed endogenously and under no atypical conditions, such as inducible stimuli including heat or stress.

**[0068]** In a specific embodiment of the present invention there is a method of identifying a compound for the treatment of a myelinopathy wherein the myelinopathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy Syndrome (RLS), comprising the steps of exposing a non-human knockout animal, wherein the animal comprises at least one defective allele of SEQ ID NO:1, to the compound; and assaying for an improvement of the myelinopathy. A skilled artisan is aware of what would be considered an improvement of a myelinopathy. For example, an improvement of myelinopathy would comprise an increase in myelin sheathing around a nerve fiber or a decrease or complete cessation of loss of myelin sheathing around a nerve fiber, or improvement of a symptom of peripheral neuropathy.

**[0069]** In a specific embodiment, a reporter sequence is utilized in the methods of the present invention. In a specific embodiment, a transgenic non-human animal comprises a reporter nucleic acid, wherein said nucleic acid is under the control of a periaxin promoter active in eukaryotic cells. A reporter sequence is a nucleic acid sequence whose expression is monitored or whose gene product a nucleic acid sequence that encodes a protein or gene product which is monitored to reflect the expression of a regulatory sequence such as a promoter. Examples of reporter sequences include histological markers such as chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), enhanced GFP, blue fluorescent protein, luciferase,  $\beta$ -galactosidase and -glucuronidase. A reporter gene containing an epitope tag can also be monitored. Examples of epitope tags include HA, myc and Flag.

**[0070]** In a specific embodiment, there is a method of diagnosing a myelinopathy in an animal, such as Charcot-Marie-Tooth disease, Dejerine-Sottas syndrome, hereditary neuropathy with liability to pressure palsies, congenital hypomyelinating neuropathy, or Roussy-Levy syndrome, comprising the step of analyzing a nucleic acid sequence of SEQ ID NO:1, wherein an alteration in the nucleic acid is associated with the myelinopathy. In another specific embodiment, the analyzing step further comprises polymerase chain reaction.

[0071] In a specific embodiment, the present invention provides a method for detecting the presence of a mutation in a periaxin polynucleotide. Detection of the presence of the mutation aids in diagnosis of a myelinopathy, such as Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy Syndrome (RLS).

[0072] In an alternative specific embodiment, the present invention provides a method for detecting the absence of a mutation in a periaxin polynucleotide. Detection of the absence of the mutation in a periaxin polynucleotide is valuable to a health care provider by narrowing the possibilities for causes of a particular phenotype such as a myelinopathy, including one associated with Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy Syndrome (RLS).

#### **B. Myelinopathy**

[0073] The methods and compositions of the present invention are directed to the following inherited peripheral neuropathies: Charcot-Marie-Tooth disease (Charcot and Marie, 1886; Tooth, 1886) types 1 and 2 (CMT1 and CMT2), also known as hereditary motor and sensory neuropathy types I and II (HMSNI and HMSNII) Dyck and Lambert, 1968a; Dyck and Lambert 1968b; Thomas et al., 1974); the Dejerine-Sottas syndrome (DSS) (Dejerine and Sottas, 1893), also known as hereditary motor and sensory neuropathy type III (HMSNIII); hereditary neuropathy with liability to pressure palsies (HNPP) (Windebank, 1993); congenital hypomyelinating neuropathy (CHN) (Harati and Butler, 1985; Charmas *et al.*, 1988); and clinical variants of CMT such as Roussy-Levy syndrome (RLS) Roussy and Levy, 1926).

[0074] Charcot-Marie-Tooth (CMT) (MIM 118220) polyneuropathy syndrome represents a clinically and genetically heterogeneous group of disorders of the peripheral nerve. Two major types are distinguished by measuring motor nerve conduction velocities (NCV). CMT1 is a demyelinating neuropathy characterized by symmetrically slowed motor NCV (usually <40 meters/second). Microscopic sections of peripheral nerve in CMT1 patients reveal onion bulb formation. CMT2 is an axonal neuropathy associated with normal or near normal NCV with decreased amplitudes and axonal loss on nerve biopsy. A skilled artisan is made aware herein that although a myelinopathy in CMT2 is not to be expected,

there is significant clinical overlap between CMT1 and CMT2, although the histopathology is usually defining. Thus, although a periaxin alteration resulting in CMT2 is unlikely, an individual having CMT2 may be tested for periaxin alterations in light of the difficulty in distinguishing between CMT1 and CMT2 clinically.

**[0075]** CMT1 which is more common and usually autosomal dominant, generally presents in the 2nd or 3rd decade, and is associated with slowly progressive symmetric distal muscle weakness and atrophy, gait disturbance, and absent stretch reflexes. CMT2 is autosomal dominant and usually manifests later in life. Different genetic subtypes of both CMT1 and CMT2 can be further delineated based on genetic linkage analysis and mapping to distinct loci.

**[0076]** Hereditary neuropathy with liability to pressure palsies (HNPP) (MIM 162500) is a demyelinating neuropathy whose neuropathological hallmark is sausage-like thickening of myelin sheaths (tomacula). Electrophysiologic findings include mildly slowed NCV and conduction blocks. The clinical manifestations are typically episodic, nonsymmetric palsies, that may be precipitated by trauma or compression. Multifocal neuropathies, especially entrapment neuropathies, such as carpal tunnel syndrome, may be manifestations of HNPP.

**[0077]** Dejerine-Sottas syndrome (DSS), or HMSNIII, was originally described as an interstitial hypertrophic neuropathy of infancy (Dejerine and Sottas, 1893). It is a more severe demyelinating neuropathy than CMT1 (Dyck *et al.*, 1993). The disease usually begins in infancy, as evidenced by delayed motor milestones, and it is generally associated with severe pathological alterations, such as more significant slowing of NCV, more pronounced demyelination, and more numerous onion bulbs than observed in CMT, and nerve conduction velocity abnormalities (<6-12 meters /sec) (Ouvrier *et al.*, 1987). The cerebrospinal fluid proteins can be elevated. Congenital hypomyelinating neuropathy (CHN) is distinguished from DSS by its congenital manifestation, and the histopathologic findings of hypomyelination and few onion bulbs.

**[0078]** Most CMT1 patients have DNA rearrangements as the molecular cause of their disease. A 1.5 Mb-tandem duplication, the CMT1A duplication, accounts for approximately 70% of CMT1 cases. A deletion of the same 1.5-Mb region in chromosome 17p12 is found in >85% of patients with HNPP. The CMT1A duplication and HNPP deletion result from unequal crossing-over and reciprocal homologous recombination involving a 24-



kb repeat, CMT1A-REP, that flanks the 1.5-Mb region. A meiotic recombination hotspot occurs within CMT1A-REP. The majority of the de novo duplication and deletion events occur in meiosis of the male germ cells.

[0079] The CMT1A and HNPP phenotypes result from a gene dosage effect. CMT1A is due to trisomic overexpression of the peripheral myelin protein-22 gene, *PMP22*, while HNPP results from monosomic underexpression of *PMP22*. In rare patients without the CMT1A duplication or HNPP deletion, *PMP22* point mutations can cause disease. Null alleles or haploinsufficiency cause HNPP, while gain-of-function or dominant-negative missense amino acid substitutions results in CMT1A or DSS.

[0080] Mutations in myelin protein zero (*MPZ*), connexin 32 (*Cx32*) or gap junction protein,  $\beta 1$  (*GJB1*), or early growth response 2 (*EGR2*, the human *Krox-20* homologue), myotubularin related protein 2 (*MTMR2*), N-myc downstream regulated gene 1 (*NDRG1*) genes can also cause CMT1 (*MPZ*, *Cx32*, *EGR2*), DSS (*MPZ*, *EGR2*), or CHN (*MPZ*, *EGR2*). Mutation of *Cx32* causes the X-linked form of CMT. Thus, these myelinopathies appear to represent a spectrum of related disorders resulting from myelin dysfunction. Each of these genes (*PMP22*, *Cx32*, *MPZ*, and *EGR2*) are expressed in myelinating Schwann cells so that mutations probably exert their effects on Schwann cells.

[0081] Clinical variability is the rule in inherited neuropathies. Discordance is even noted in identical twins with the CMT1A duplication. *De novo* CMT1A duplication is frequently found in sporadic CMT1. DSS and RLS can also be associated with CMT1A duplication. Multifocal neuropathy, autosomal dominant carpal tunnel syndrome, and CMT1 can also be associated with the HNPP deletion. These inherited demyelinating neuropathies can be difficult to distinguish from acquired demyelinating neuropathies. Because of the clinical heterogeneity, the clinical workup of a patient with peripheral neuropathy requires molecular definition. Determining an exact molecular etiology enables a precise and secure diagnosis, provides prognostic information, allows proper genetic counseling, and makes possible the design and implementation of rational therapeutic strategies.

[0082] Congenital hypomyelinating neuropathy (CHN) is characterized by infantile hypotonia, distal muscle weakness, areflexia, and markedly slow NCVs (<10 meters/sec). In severe cases, joint contractures or arthrogryposis multiplex congenita have been described (Charmas et al., 1988). In less severe cases it is difficult to differentiate CHN from DSS. Some have considered both DSS and CHN as forms of HMSNIII Dyck *et al.*, 1993). The

nerve biopsies show hypomyelination (few thin myelin lamellae) without active myelin breakdown products and early onion bulb formations. However, there are several histological phenotypes for DSS and CHN (Harati and Butler, 1985).

[0083] Roussy-Levy syndrome (RLS) was described in patients presenting with *pes cavus*, distal limb weakness, areflexia, distal sensory loss, sensory gait ataxia, and tremor (Roussy and Levy, 1926). It is controversial whether RLS represents a clinical entity distinct from CMT or a clinical variant.

### C. The Periaxin Gene

[0084] The periaxin gene (*PRX*) encodes two PDZ domain proteins, L- and S-periaxin, that are required for the maintenance of peripheral nerve myelin. In murine embryonic Schwann cells, L-periaxin is initially concentrated in the nuclei but redistributes to the adaxonal plasma membrane with initiation of myelination and then to the abaxonal, Schmidt-Lanterman incisure, and paranodal membranes with maturation of the myelin sheath (Scherer *et al.*, 1995; Sherman and Brophy, 2000). L-periaxin expression recapitulates this pattern following nerve crush injury (Scherer *et al.*, 1995). This shift in periaxin localization during myelination suggests that periaxin participates in membrane-protein interactions that are required to stabilize the mature myelin sheath. As a cytoskeleton-associated protein, L-periaxin in some embodiments mediates such stabilization by facilitating integration of extracellular signaling through the cytoskeleton, a function essential for changes in Schwann cell shape and regulation of gene expression during axonal ensheathment (Fernandez-Valle *et al.*, 1997; Tapon *et al.*, 1997). Such a signaling function is supported by two observations: first, L-periaxin contains a nuclear localization signal and a PDZ motif, a domain implicated in the assembly of signaling complexes at sites of cell-cell contact (Sherman and Brophy, 2000; Dytrych *et al.*, 1998); and second L-periaxin binds dystroglycan-dystrophin-related protein 2 (DRP2) which is part of a complex linking extracellular matrix proteins to the cytoskeleton and cortical signaling molecules (Sherman *et al.*, 2001). Confirming the necessity of periaxin for maintenance of the myelin sheath, Gillespie *et al* demonstrated that *Prx*<sup>-/-</sup> mice ensheath and myelinate peripheral axons normally but subsequently develop a severe demyelinating neuropathy associated with allodynia (pain from normally non-noxious stimuli) and hyperalgesia (hypersensitivity to painful stimuli) (Gillespie *et al.*, 2000).

### D. Nucleic Acid-Based Expression Systems

#### 1. Vectors

[0085] In specific methods of the present invention, a vector is utilized to transport an exogenous nucleic acid sequence. A nucleic acid sequence is "exogenous," if it is foreign to the cell into which the vector is being introduced or if the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs)). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques, which are described in Maniatis *et al.*, 1988 and Ausubel *et al.*, 1994, both incorporated herein by reference.

[0086] The term "expression vector" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described *infra*.

**a. Promoters and Enhancers**

[0087] A "promoter" is a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases "operatively positioned," "operatively linked," "under control," and "under transcriptional control" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence. A promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0088] A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a nucleic acid sequence, located either downstream or

upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," *i.e.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein (see U.S. Patent 4,683,202, U.S. Patent 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

[0089] Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression, for example, see Sambrook *et al.* (1989), incorporated herein by reference. The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

[0090] The identity of tissue-specific promoters or elements, as well as assays to characterize their activity, is well known to those of skill in the art. Examples of such regions include the human LIMK2 gene (Nomoto *et al.* 1999), the somatostatin receptor 2 gene (Kraus *et al.*, 1998), murine epididymal retinoic acid-binding gene (Lareyre *et al.*, 1999), human CD4 (Zhao-Emonet *et al.*, 1998), mouse alpha2 (XI) collagen (Tsumaki, *et al.*, 1998), D1A dopamine receptor gene (Lee, *et al.*, 1997), insulin-like growth factor II (Wu *et al.*, 1997), human platelet endothelial cell adhesion molecule-1 (Almendo *et al.*, 1996).

#### **b. Initiation Signals and Internal Ribosome Binding Sites**

[0091] A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be "in-frame" with the reading frame of the desired coding sequence to ensure translation of the entire insert. The exogenous translational control signals and initiation codons can be either natural or synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements.

[0092] In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5' methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988). IRES elements from two members of the picornavirus family (polio and encephalomyocarditis) have been described (Pelletier and Sonenberg, 1988), as well an IRES from a mammalian message (Macejak and Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Patent 5,925,565 and 5,935,819, herein incorporated by reference).

### c. Multiple Cloning Sites

[0093] Vectors can include a multiple cloning site (MCS), which is a nucleic acid region that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector. (See Carbonelli *et al.*, 1999, Levenson *et al.*, 1998, and Cocca, 1997, incorporated herein by reference.) "Restriction enzyme digestion" refers to catalytic cleavage of a nucleic acid molecule with an enzyme that functions only at specific locations in a nucleic acid molecule. Many of these restriction enzymes are commercially available. Use of such enzymes is widely understood by those of skill in the art. Frequently, a vector is linearized or fragmented using a restriction enzyme that cuts within the MCS to enable exogenous sequences to be ligated to the vector. "Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid

fragments, which may or may not be contiguous with each other. Techniques involving restriction enzymes and ligation reactions are well known to those of skill in the art of recombinant technology.

**d. Splicing Sites**

[0094] Most transcribed eukaryotic RNA molecules will undergo RNA splicing to remove introns from the primary transcripts. Vectors containing genomic eukaryotic sequences may require donor and/or acceptor splicing sites to ensure proper processing of the transcript for protein expression. (See Chandler *et al.*, 1997, herein incorporated by reference.)

**e. Polyadenylation Signals**

[0095] In expression, one will typically include a polyadenylation signal to effect proper polyadenylation of the transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and/or any such sequence may be employed. Preferred embodiments include the SV40 polyadenylation signal and/or the bovine growth hormone polyadenylation signal, convenient and/or known to function well in various target cells. Also contemplated as an element of the expression cassette is a transcriptional termination site. These elements can serve to enhance message levels and/or to minimize read through from the cassette into other sequences.

**f. Origins of Replication**

[0096] In order to propagate a vector in a host cell, it may contain one or more origins of replication sites (often termed "ori"), which is a specific nucleic acid sequence at which replication is initiated. Alternatively an autonomously replicating sequence (ARS) can be employed if the host cell is yeast.

**g. Selectable and Screenable Markers**

[0097] In certain embodiments of the invention, the cells contain nucleic acid construct of the present invention, a cell may be identified *in vitro* or *in vivo* by including a marker in the expression vector. Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the recombinant vector. Generally, a selectable marker is one that confers a property that allows for selection. A positive selectable marker is one in which the presence of the marker allows for its selection, while a

negative selectable marker is one in which its presence prevents its selection. An example of a positive selectable marker is a drug resistance marker.

[0098] Usually the inclusion of a drug selection marker aids in the cloning and identification of transformants, for example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selectable markers. In addition to markers conferring a phenotype that allows for the discrimination of transformants based on the implementation of conditions, other types of markers including screenable markers such as GFP, whose basis is colorimetric analysis, are also contemplated. Alternatively, screenable enzymes such as herpes simplex virus thymidine kinase (*tk*) or chloramphenicol acetyltransferase (CAT) may be utilized. One of skill in the art would also know how to employ immunologic markers, possibly in conjunction with FACS analysis. The marker used is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid of the present application encoding a gene product or a portion thereof. Further examples of selectable and screenable markers are well known to one of skill in the art.

## 2. Host Cells

[0099] As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. All of these term also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell, and it includes any transformable organisms that is capable of replicating a vector and/or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors. A host cell may be "transfected" or "transformed," which refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

[0100] Host cells may be derived from prokaryotes or eukaryotes, depending upon whether the desired result is replication of the vector or expression of part or all of the vector-encoded nucleic acid sequences. Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials ([www.atcc.org](http://www.atcc.org)). An appropriate host can be determined by one of skill in the art based on the

vector backbone and the desired result. A plasmid or cosmid, for example, can be introduced into a prokaryotic host cell for replication of many vectors. Bacterial cells used as host cells for vector replication and/or expression include *E. coli* K12, DH5 $\alpha$ , JM109, and KC8 strains, as well as a number of commercially available bacterial hosts such as SURE<sup>®</sup> Competent Cells and SOLOPACK<sup>™</sup> Gold Cells (STRATAGENE<sup>®</sup>, La Jolla). Alternatively, bacterial cells such as *E. coli* K12 or LE392 strains could be used as host cells for phage viruses.

[0101] Examples of eukaryotic host cells for replication and/or expression of a vector include HeLa, NIH3T3, Jurkat, 293, Cos, CHO, Saos, and PC12. Many host cells from various cell types and organisms are available and would be known to one of skill in the art. Similarly, a viral vector may be used in conjunction with either a eukaryotic or prokaryotic host cell, particularly one that is permissive for replication or expression of the vector.

[0102] Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. One of skill in the art would further understand the conditions under which to incubate all of the above described host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

### 3. Expression Systems

[0103] Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with the present invention to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Many such systems are commercially and widely available.

[0104] The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Patent No. 5,871,986, 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MAXBAC<sup>®</sup> 2.0 from INVITROGEN<sup>®</sup> and BACPACK<sup>™</sup> BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH<sup>®</sup>.

[0105] Other examples of expression systems include STRATAGENE<sup>®</sup>'s COMPLETE CONTROL<sup>™</sup> Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an *E. coli* expression system. Another



example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN® also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast *Pichia methanolica*. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

#### **E. Nucleic Acid Detection**

[0106] In addition to their use in directing the expression of periaxin proteins, polypeptides and/or peptides, the nucleic acid sequences disclosed herein have a variety of other uses. For example, they have utility as probes or primers for embodiments involving nucleic acid hybridization.

##### **1. Hybridization**

[0107] The use of a probe or primer of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length, or in some aspects of the invention up to 1-2 kilobases or more in length, allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over contiguous stretches greater than 20 bases in length are generally preferred, to increase stability and/or selectivity of the hybrid molecules obtained. One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

[0108] Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of DNAs and/or RNAs or to provide primers for amplification of DNA or RNA from samples. Depending on the application envisioned, one would desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of the probe or primers for the target sequence.

[0109] For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl

at temperatures of about 50°C to about 70°C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0110] For certain applications, for example, site-directed mutagenesis, it is appreciated that lower stringency conditions are preferred. Under these conditions, hybridization may occur even though the sequences of the hybridizing strands are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and/or decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37°C to about 55°C, while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Hybridization conditions can be readily manipulated depending on the desired results.

[0111] In other embodiments, hybridization may be achieved under conditions of, for example, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl<sub>2</sub>, 1.0 mM dithiothreitol, at temperatures between approximately 20°C to about 37°C. Other hybridization conditions utilized could include approximately 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, at temperatures ranging from approximately 40°C to about 72°C.

[0112] In certain embodiments, it will be advantageous to employ nucleic acids of defined sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of being detected. In preferred embodiments, one may desire to employ a fluorescent label or an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a detection means that is visibly or spectrophotometrically detectable, to identify specific hybridization with complementary nucleic acid containing samples.

[0113] In general, it is envisioned that the probes or primers described herein will be useful as reagents in solution hybridization, as in PCR™, for detection of expression of

corresponding genes, as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to hybridization with selected probes under desired conditions. The conditions selected will depend on the particular circumstances (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Optimization of hybridization conditions for the particular application of interest is well known to those of skill in the art. After washing of the hybridized molecules to remove non-specifically bound probe molecules, hybridization is detected, and/or quantified, by determining the amount of bound label. Representative solid phase hybridization methods are disclosed in U.S. Patent Nos. 5,843,663, 5,900,481 and 5,919,626. Other methods of hybridization that may be used in the practice of the present invention are disclosed in U.S. Patent Nos. 5,849,481, 5,849,486 and 5,851,772. The relevant portions of these and other references identified in this section of the Specification are incorporated herein by reference.

## **2. Amplification of Nucleic Acids**

[0114] Nucleic acids used as a template for amplification may be isolated from cells, tissues or other samples according to standard methodologies (Sambrook *et al.*, 1989). In certain embodiments, analysis is performed on whole cell or tissue homogenates or biological fluid samples without substantial purification of the template nucleic acid. The nucleic acid may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to first convert the RNA to a complementary DNA.

[0115] The term "primer," as used herein, is meant to encompass any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process. Typically, primers are oligonucleotides from ten to twenty and/or thirty base pairs in length, but longer sequences can be employed. Primers may be provided in double-stranded and/or single-stranded form, although the single-stranded form is preferred.

[0116] Pairs of primers designed to selectively hybridize to nucleic acids corresponding to SEQ ID NO:1 or to at least one of SEQ ID NO:27 through SEQ ID NO:53 are contacted with the template nucleic acid under conditions that permit selective hybridization. Depending upon the desired application, high stringency hybridization conditions may be selected that will only allow hybridization to sequences that are completely complementary to the primers. In other embodiments, hybridization may occur

under reduced stringency to allow for amplification of nucleic acids that contain one or more mismatches with the primer sequences. Once hybridized, the template-primer complex is contacted with one or more enzymes that facilitate template-dependent nucleic acid synthesis. Multiple rounds of amplification, also referred to as "cycles," are conducted until a sufficient amount of amplification product is produced.

[0117] The amplification product may be detected or quantified. In certain applications, the detection may be performed by visual means. Alternatively, the detection may involve indirect identification of the product via chemiluminescence, radioactive scintigraphy of incorporated radiolabel or fluorescent label or even via a system using electrical and/or thermal impulse signals (Affymax technology; Bellus, 1994).

[0118] A number of template dependent processes are available to amplify the oligonucleotide sequences present in a given template sample. One of the best known amplification methods is the polymerase chain reaction (referred to as PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, and in Innis *et al.*, 1990, each of which is incorporated herein by reference in their entirety.

[0119] A reverse transcriptase PCR™ (RT-PCR) amplification procedure may be performed to quantify the amount of mRNA amplified. Methods of reverse transcribing RNA into cDNA are well known and described in Sambrook *et al.*, 1989. Alternative methods for reverse transcription utilize thermostable DNA polymerases. These methods are described in WO 90/07641. Polymerase chain reaction methodologies are well known in the art. Representative methods of RT-PCR are described in U.S. Patent No. 5,882,864.

[0120] Another method for amplification is ligase chain reaction ("LCR"), disclosed in European Application No. 320 308, incorporated herein by reference in its entirety. U.S. Patent 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence. A method based on PCR™ and oligonucleotide ligase assay (OLA), disclosed in U.S. Patent 5,912,148, may also be used.

[0121] Alternative methods for amplification of target nucleic acid sequences that may be used in the practice of the present invention are disclosed in U.S. Patent Nos. 5,843,650, 5,846,709, 5,846,783, 5,849,546, 5,849,497, 5,849,547, 5,858,652, 5,866,366, 5,916,776, 5,922,574, 5,928,905, 5,928,906, 5,932,451, 5,935,825, 5,939,291 and 5,942,391, GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety.

[0122] Qbeta Replicase, described in PCT Application No. PCT/US87/00880, may also be used as an amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence which may then be detected.

[0123] An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[alpha-thio]-triphosphates in one strand of a restriction site may also be useful in the amplification of nucleic acids in the present invention (Walker *et al.*, 1992). Strand Displacement Amplification (SDA), disclosed in U.S. Patent No. 5,916,779, is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.*, nick translation.

[0124] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh *et al.*, 1989; Gingeras *et al.*, PCT Application WO 88/10315, incorporated herein by reference in their entirety). Davey *et al.*, European Application No. 329 822 disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention.

[0125] Miller *et al.*, PCT Application WO 89/06700 (incorporated herein by reference in its entirety) disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter region/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic, *i.e.*, new templates are not produced from the resultant RNA transcripts. Other amplification methods include "race" and "one-sided PCR" (Frohman, 1990; Ohara *et al.*, 1989).

### 3. Detection of Nucleic Acids

[0126] Following any amplification, it may be desirable to separate the amplification product from the template and/or the excess primer. In one embodiment, amplification products are separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using standard methods (Sambrook *et al.*, 1989). Separated amplification products may be cut out and eluted from the gel for further manipulation. Using low melting point agarose

gels, the separated band may be removed by heating the gel, followed by extraction of the nucleic acid.

[0127] Separation of nucleic acids may also be effected by chromatographic techniques known in art. There are many kinds of chromatography which may be used in the practice of the present invention, including adsorption, partition, ion-exchange, hydroxylapatite, molecular sieve, reverse-phase, column, paper, thin-layer, and gas chromatography as well as HPLC.

[0128] In certain embodiments, the amplification products are visualized. A typical visualization method involves staining of a gel with ethidium bromide and visualization of bands under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the separated amplification products can be exposed to x-ray film or visualized under the appropriate excitatory spectra.

[0129] In one embodiment, following separation of amplification products, a labeled nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, or another binding partner carrying a detectable moiety.

[0130] In particular embodiments, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art. See Sambrook *et al.*, 1989. One example of the foregoing is described in U.S. Patent No. 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

[0131] Other methods of nucleic acid detection that may be used in the practice of the instant invention are disclosed in U.S. Patent Nos. 5,840,873, 5,843,640, 5,843,651, 5,846,708, 5,846,717, 5,846,726, 5,846,729, 5,849,487, 5,853,990, 5,853,992, 5,853,993, 5,856,092, 5,861,244, 5,863,732, 5,863,753, 5,866,331, 5,905,024, 5,910,407, 5,912,124, 5,912,145, 5,919,630, 5,925,517, 5,928,862, 5,928,869, 5,929,227, 5,932,413 and 5,935,791, each of which is incorporated herein by reference.

#### 4. Other Assays

[0132] Other methods for genetic screening may be used within the scope of the present invention, for example, to detect mutations in genomic DNA, cDNA and/or RNA samples. Methods used to detect point mutations include denaturing gradient gel electrophoresis ("DGGE"), restriction fragment length polymorphism analysis ("RFLP"), chemical or enzymatic cleavage methods, direct sequencing of target regions amplified by PCR<sup>TM</sup> (see above), single-strand conformation polymorphism analysis ("SSCP"), denaturing high pressure liquid chromatography (DHPLC) and other methods well known in the art.

[0133] One method of screening for point mutations is based on RNase cleavage of base pair mismatches in RNA/DNA or RNA/RNA heteroduplexes. As used herein, the term "mismatch" is defined as a region of one or more unpaired or mispaired nucleotides in a double-stranded RNA/RNA, RNA/DNA or DNA/DNA molecule. This definition thus includes mismatches due to insertion/deletion mutations, as well as single or multiple base point mutations.

[0134] U.S. Patent No. 4,946,773 describes an RNase A mismatch cleavage assay that involves annealing single-stranded DNA or RNA test samples to an RNA probe, and subsequent treatment of the nucleic acid duplexes with RNase A. For the detection of mismatches, the single-stranded products of the RNase A treatment, electrophoretically separated according to size, are compared to similarly treated control duplexes. Samples containing smaller fragments (cleavage products) not seen in the control duplex are scored as positive.

[0135] Other investigators have described the use of RNase I in mismatch assays. The use of RNase I for mismatch detection is described in literature from Promega Biotech. Promega markets a kit containing RNase I that is reported to cleave three out of four known mismatches. Others have described using the MutS protein or other DNA-repair enzymes for detection of single-base mismatches.

[0136] Alternative methods for detection of deletion, insertion or substitution mutations that may be used in the practice of the present invention are disclosed in U.S. Patent Nos. 5,849,483, 5,851,770, 5,866,337, 5,925,525 and 5,928,870, each of which is incorporated herein by reference in its entirety.

## 5. Kits

[0137] All the essential materials and/or reagents required for detecting a periaxin nucleic acid sequence in a sample may be assembled together in a kit. This generally will

comprise a probe or primers designed to hybridize specifically to individual nucleic acids of interest in the practice of the present invention, including SEQ ID NO:1 and/or SEQ ID NO:27 through SEQ ID NO:53. Also included may be enzymes suitable for amplifying nucleic acids, including various polymerases (reverse transcriptase, *Taq*, etc.), deoxynucleotides and buffers to provide the necessary reaction mixture for amplification. Such kits may also include enzymes and other reagents suitable for detection of specific nucleic acids or amplification products. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or enzyme as well as for each probe or primer pair.

## **F. Periaxin Nucleic Acids**

### **1. Nucleic Acids and Uses Thereof**

[0138] Certain aspects of the present invention concern at least one periaxin nucleic acid. In certain aspects, the at least one periaxin nucleic acid comprises a wild-type or mutant periaxin nucleic acid. In particular aspects, the periaxin nucleic acid encodes for at least one transcribed nucleic acid. In particular aspects, the periaxin nucleic acid encodes at least one periaxin protein, polypeptide or peptide, or biologically functional equivalent thereof. In other aspects, the periaxin nucleic acid comprises at least one nucleic acid segment of SEQ ID NO:1 or one of SEQ ID NO:27 through SEQ ID NO:53, or at least one biologically functional equivalent thereof.

[0139] The present invention also concerns the isolation or creation of at least one recombinant construct or at least one recombinant host cell through the application of recombinant nucleic acid technology known to those of skill in the art or as described herein. The recombinant construct or host cell may comprise at least one periaxin nucleic acid, and may express at least one periaxin protein, polypeptide or peptide, or at least one biologically functional equivalent thereof.

[0140] As used herein "wild-type" refers to the naturally occurring sequence of a nucleic acid at a genetic locus in the genome of an organism, and sequences transcribed or translated from such a nucleic acid. Thus, the term "wild-type" also may refer to the amino acid sequence encoded by the nucleic acid. As a genetic locus may have more than one sequence or alleles in a population of individuals, the term "wild-type" encompasses all such naturally occurring alleles. As used herein the term "polymorphic" means that variation exists (*i.e.* two or more alleles exist) at a genetic locus in the individuals of a population. As



used herein "mutant" refers to a change in the sequence of a nucleic acid or its encoded protein, polypeptide or peptide that is the result of the hand of man.

[0141] A nucleic acid may be made by any technique known to one of ordinary skill in the art. Non-limiting examples of synthetic nucleic acid, particularly a synthetic oligonucleotide, include a nucleic acid made by *in vitro* chemical synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266,032, incorporated herein by reference, or *via* deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, 1986, and U.S. Patent Serial No. 5,705,629, each incorporated herein by reference. A non-limiting example of enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCR<sup>TM</sup> (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195, each incorporated herein by reference), or the synthesis of oligonucleotides described in U.S. Patent No. 5,645,897, incorporated herein by reference. A non-limiting example of a biologically produced nucleic acid includes recombinant nucleic acid production in living cells, such as recombinant DNA vector production in bacteria (see for example, Sambrook *et al.* 1989, incorporated herein by reference).

[0142] A nucleic acid may be purified on polyacrylamide gels, cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook *et al.* 1989, incorporated herein by reference).

[0143] The term "nucleic acid" will generally refer to at least one molecule or strand of DNA, RNA or a derivative or mimic thereof, comprising at least one nucleobase, such as, for example, a naturally occurring purine or pyrimidine base found in DNA (*e.g.* adenine "A," guanine "G," thymine "T" and cytosine "C") or RNA (*e.g.* A, G, uracil "U" and C). The term "nucleic acid" encompass the terms "oligonucleotide" and "polynucleotide." The term "oligonucleotide" refers to at least one molecule of between about 3 and about 100 nucleobases in length. The term "polynucleotide" refers to at least one molecule of greater than about 100 nucleobases in length. These definitions generally refer to at least one single-stranded molecule, but in specific embodiments will also encompass at least one additional strand that is partially, substantially or fully complementary to the at least one single-stranded molecule. Thus, a nucleic acid may encompass at least one double-stranded molecule or at least one triple-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence comprising a strand of the molecule. As used

herein, a single stranded nucleic acid may be denoted by the prefix "ss", a double stranded nucleic acid by the prefix "ds", and a triple stranded nucleic acid by the prefix "ts."

**[0144]** Thus, the present invention also encompasses at least one nucleic acid that is complementary to a periaxin nucleic acid. In particular embodiments, the invention encompasses at least one nucleic acid or nucleic acid segment complementary to the sequence set forth in SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:53. Nucleic acid(s) that are "complementary" or "complement(s)" are those that are capable of base-pairing according to the standard Watson-Crick, Hoogsteen or reverse Hoogsteen binding complementarity rules. As used herein, the term "complementary" or "complement(s)" also refers to nucleic acid(s) that are substantially complementary, as may be assessed by the same nucleotide comparison set forth above. The term "substantially complementary" refers to a nucleic acid comprising at least one sequence of consecutive nucleobases, or semiconsecutive nucleobases if one or more nucleobase moieties are not present in the molecule, are capable of hybridizing to at least one nucleic acid strand or duplex even if less than all nucleobases do not base pair with a counterpart nucleobase. In certain embodiments, a "substantially complementary" nucleic acid contains at least one sequence in which about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, to about 100%, and any range therein, of the nucleobase sequence is capable of base-pairing with at least one single or double stranded nucleic acid molecule during hybridization. In certain embodiments, the term "substantially complementary" refers to at least one nucleic acid that may hybridize to at least one nucleic acid strand or duplex in stringent conditions. In certain embodiments, a "partly complementary" nucleic acid comprises at least one sequence that may hybridize in low stringency conditions to at least one single or double stranded nucleic acid, or contains at least one sequence in which less than about 70% of the nucleobase sequence is capable of base-pairing with at least one single or double stranded nucleic acid molecule during hybridization.

**[0145]** As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

[0146] As used herein "stringent condition(s)" or "high stringency" are those that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating at least one nucleic acid, such as a gene or nucleic acid segment thereof, or detecting at least one specific mRNA transcript or nucleic acid segment thereof, and the like.

[0147] Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence of formamide, tetramethylammonium chloride or other solvent(s) in the hybridization mixture. It is generally appreciated that conditions may be rendered more stringent, such as, for example, the addition of increasing amounts of formamide.

[0148] It is also understood that these ranges, compositions and conditions for hybridization are mentioned by way of non-limiting example only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to employ varying conditions of hybridization to achieve varying degrees of selectivity of the nucleic acid(s) towards target sequence(s). In a non-limiting example, identification or isolation of related target nucleic acid(s) that do not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions", and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M NaCl at a temperature range of about 20°C to about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suite a particular application.

[0149] One or more nucleic acid(s) may comprise, or be composed entirely of, at least one derivative or mimic of at least one nucleobase, a nucleobase linker moiety and/or backbone moiety that may be present in a naturally occurring nucleic acid. As used herein a

"derivative" refers to a chemically modified or altered form of a naturally occurring molecule, while the terms "mimic" or "analog" refers to a molecule that may or may not structurally resemble a naturally occurring molecule, but functions similarly to the naturally occurring molecule. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure, and is encompassed by the term "molecule."

**[0150]** As used herein a "nucleobase" refers to a naturally occurring heterocyclic base, such as A, T, G, C or U ("naturally occurring nucleobase(s)"), found in at least one naturally occurring nucleic acid (i.e. DNA and RNA), and their naturally or non-naturally occurring derivatives and mimics. Non-limiting examples of nucleobases include purines and pyrimidines, as well as derivatives and mimics thereof, which generally can form one or more hydrogen bonds ("anneal" or "hybridize") with at least one naturally occurring nucleobase in manner that may substitute for naturally occurring nucleobase pairing (e.g. the hydrogen bonding between A and T, G and C, and A and U).

**[0151]** Nucleobase, nucleoside and nucleotide mimics or derivatives are well known in the art, and have been described in exemplary references such as, for example, Scheit, Nucleotide Analogs (John Wiley, New York, 1980), incorporated herein by reference. "Purine" and "pyrimidine" nucleobases encompass naturally occurring purine and pyrimidine nucleobases and also derivatives and mimics thereof, including but not limited to, those purines and pyrimidines substituted by one or more of alkyl, carboxyalkyl, amino, hydroxyl, halogen (i.e. fluoro, chloro, bromo, or iodo), thiol, or alkylthiol wherein the alkyl group comprises of from about 1, about 2, about 3, about 4, about 5, to about 6 carbon atoms. Non-limiting examples of purines and pyrimidines include deazapurines, 2,6-diaminopurine, 5-fluorouracil, xanthine, hypoxanthine, 8-bromoguanine, 8-chloroguanine, bromothymine, 8-aminoguanine, 8-hydroxyguanine, 8-methylguanine, 8-thioguanine, azaguanines, 2-aminopurine, 5-ethylcytosine, 5-methylcytosine, 5-bromouracil, 5-ethyluracil, 5-iodouracil, 5-chlorouracil, 5-propyluracil, thiouracil, 2-methyladenine, methylthioadenine, N,N-dimethyladenine, azaadenines, 8-bromoadenine, 8-hydroxyadenine, 6-hydroxyaminopurine, 6-thiopurine, 4-(6-aminoethyl/cytosine), and the like. A table of exemplary, but not limiting, purine and pyrimidine derivatives and mimics is also provided herein below.

**[0152]** As used herein, "nucleoside" refers to an individual chemical unit comprising a nucleobase covalently attached to a nucleobase linker moiety. A non-limiting example of a

"nucleobase linker moiety" is a sugar comprising 5-carbon atoms (a "5-carbon sugar"), including but not limited to deoxyribose, ribose or arabinose, and derivatives or mimics of 5-carbon sugars. Non-limiting examples of derivatives or mimics of 5-carbon sugars include 2'-fluoro-2'-deoxyribose or carbocyclic sugars where a carbon is substituted for the oxygen atom in the sugar ring. By way of non-limiting example, nucleosides comprising purine (*i.e.* A and G) or 7-deazapurine nucleobases typically covalently attach the 9 position of the purine or 7-deazapurine to the 1'-position of a 5-carbon sugar. In another non-limiting example, nucleosides comprising pyrimidine nucleobases (*i.e.* C, T or U) typically covalently attach the 1 position of the pyrimidine to 1'-position of a 5-carbon sugar (Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992). However, other types of covalent attachments of a nucleobase to a nucleobase linker moiety are known in the art, and non-limiting examples are described herein.

[0153] As used herein, a "nucleotide" refers to a nucleoside further comprising a "backbone moiety" generally used for the covalent attachment of one or more nucleotides to another molecule or to each other to form one or more nucleic acids. The "backbone moiety" in naturally occurring nucleotides typically comprises a phosphorus moiety, which is covalently attached to a 5-carbon sugar. The attachment of the backbone moiety typically occurs at either the 3'- or 5'-position of the 5-carbon sugar. However, other types of attachments are known in the art, particularly when the nucleotide comprises derivatives or mimics of a naturally occurring 5-carbon sugar or phosphorus moiety, and non-limiting examples are described herein.

[0154] A non-limiting example of a nucleic acid comprising such nucleoside or nucleotide derivatives and mimics is a "polyether nucleic acid", described in U.S. Patent Serial No. 5,908,845, incorporated herein by reference, wherein one or more nucleobases are linked to chiral carbon atoms in a polyether backbone. Another example of a nucleic acid comprising nucleoside or nucleotide derivatives or mimics is a "peptide nucleic acid", also known as a "PNA", "peptide-based nucleic acid mimics" or "PENAMs", described in U.S. Patent Serial Nos. 5,786,461, 5,891,625, 5,773,571, 5,766,855, 5,736,336, 5,719,262, 5,714,331, 5,539,082, and WO 92/20702, each of which is incorporated herein by reference. A peptide nucleic acid generally comprises at least one nucleobase and at least one nucleobase linker moiety that is either not a 5-carbon sugar and/or at least one backbone moiety that is not a phosphate backbone moiety. Examples of nucleobase linker moieties

described for PNAs include aza nitrogen atoms, amido and/or ureido tethers (see for example, U.S. Patent No. 5,539,082). Examples of backbone moieties described for PNAs include an aminoethylglycine, polyamide, polyethyl, polythioamide, polysulfonamide or polysulfonamide backbone moiety.

**[0155]** Peptide nucleic acids generally have enhanced sequence specificity, binding properties, and resistance to enzymatic degradation in comparison to molecules such as DNA and RNA (Egholm et al., Nature 1993, 365, 566; PCT/EP/01219). In addition, U.S. Patent Nos. 5,766,855, 5,719,262, 5,714,331 and 5,736,336 describe PNAs comprising naturally and non-naturally occurring nucleobases and alkylamine side chains with further improvements in sequence specificity, solubility and binding affinity. These properties promote double or triple helix formation between a target nucleic acid and the PNA.

**[0156]** U.S. Patent No. 5,641,625 describes that the binding of a PNA to a target sequence has applications including the creation of PNA probes to nucleotide sequences, modulating (*i.e.* enhancing or reducing) gene expression by binding of a PNA to an expressed nucleotide sequence, and cleavage of specific dsDNA molecules. In certain embodiments, nucleic acid analogues such as one or more peptide nucleic acids may be used to inhibit nucleic acid amplification, such as in PCR, to reduce false positives and discriminate between single base mutants, as described in U.S. Patent Serial No. 5891,625.

**[0157]** U.S. Patent 5,786,461 describes PNAs with amino acid side chains attached to the PNA backbone to enhance solubility. The neutrality of the PNA backbone may contribute to the thermal stability of PNA/DNA and PNA/RNA duplexes by reducing charge repulsion. The melting temperature of PNA containing duplexes, or temperature at which the strands of the duplex release into single stranded molecules, has been described as less dependent upon salt concentration.

**[0158]** One method for increasing amount of cellular uptake property of PNAs is to attach a lipophilic group. U.S. application Ser. No. 117,363, filed Sep. 3, 1993, describes several alkylamino functionalities and their use in the attachment of such pendant groups to oligonucleotides. U.S. application Ser. No. 07/943,516, filed Sep. 11, 1992, and its corresponding published PCT application WO 94/06815, describe other novel amine-containing compounds and their incorporation into oligonucleotides for, *inter alia*, the purposes of enhancing cellular uptake, increasing lipophilicity, causing greater cellular retention and increasing the distribution of the compound within the cell.

[0159] Additional non-limiting examples of nucleosides, nucleotides or nucleic acids comprising 5-carbon sugar and/or backbone moiety derivatives or mimics are well known in the art.

[0160] In certain aspect, the present invention concerns at least one nucleic acid that is an isolated nucleic acid. As used herein, the term "isolated nucleic acid" refers to at least one nucleic acid molecule that has been isolated free of, or is otherwise free of, the bulk of the total genomic and transcribed nucleic acids of one or more cells, particularly mammalian cells, and more particularly human and/or mouse and/or rat cells. In certain embodiments, "isolated nucleic acid" refers to a nucleic acid that has been isolated free of, or is otherwise free of, bulk of cellular components and macromolecules such as lipids, proteins, small biological molecules, and the like. As different species may have a RNA or a DNA containing genome, the term "isolated nucleic acid" encompasses both the terms "isolated DNA" and "isolated RNA". Thus, the isolated nucleic acid may comprise a RNA or DNA molecule isolated from, or otherwise free of, the bulk of total RNA, DNA or other nucleic acids of a particular species. As used herein, an isolated nucleic acid isolated from a particular species is referred to as a "species specific nucleic acid." When designating a nucleic acid isolated from a particular species, such as human, such a type of nucleic acid may be identified by the name of the species. For example, a nucleic acid isolated from one or more humans would be an "isolated human nucleic acid", a nucleic acid isolated from human would be an "isolated human nucleic acid", and so forth.

[0161] Of course, more than one copy of an isolated nucleic acid may be isolated from biological material, or produced in vitro, using standard techniques that are known to those of skill in the art. In particular embodiments, the isolated nucleic acid is capable of expressing a protein, polypeptide or peptide that has periaxin activity. In other embodiments, the isolated nucleic acid comprises an isolated periaxin gene.

[0162] In certain embodiments, a "gene" refers to a nucleic acid that is transcribed. As used herein, a "gene segment" is a nucleic acid segment of a gene. In certain aspects, the gene includes regulatory sequences involved in transcription, or message production or composition. In particular embodiments, the gene comprises transcribed sequences that encode for a protein, polypeptide or peptide. In other particular aspects, the gene comprises a periaxin nucleic acid, and/or encodes a periaxin polypeptide or peptide coding sequences. The term "an amino acid sequence" as used herein may be used interchangeably with the

terms protein, polypeptide, or peptide, and the like. In keeping with the terminology described herein, an "isolated gene" may comprise transcribed nucleic acid(s), regulatory sequences, coding sequences, or the like, isolated substantially away from other such sequences, such as other naturally occurring genes, regulatory sequences, polypeptide or peptide encoding sequences, and so forth. In this respect, the term "gene" is used for simplicity to refer to a nucleic acid comprising a nucleotide sequence that is transcribed, and the complement thereof. In particular aspects, the transcribed nucleotide sequence comprises at least one functional protein, polypeptide and/or peptide encoding unit. As will be understood by those in the art, this function term "gene" includes both genomic sequences, RNA or cDNA sequences or smaller engineered nucleic acid segments, including nucleic acid segments of a non-transcribed part of a gene, including but not limited to the non-transcribed promoter or enhancer regions of a gene. Smaller engineered gene nucleic acid segments may express, or may be adapted to express using nucleic acid manipulation technology, proteins, polypeptides, domains, peptides, fusion proteins, mutants and/or such like.

**[0163]** "Isolated substantially away from other coding sequences" means that the gene of interest, in this case the periaxin gene(s), forms the significant part of the coding region of the nucleic acid, or that the nucleic acid does not contain large portions of naturally-occurring coding nucleic acids, such as large chromosomal fragments, other functional genes, RNA or cDNA coding regions. Of course, this refers to the nucleic acid as originally isolated, and does not exclude genes or coding regions later added to the nucleic acid by the hand of man.

**[0164]** In certain embodiments, the nucleic acid is a nucleic acid segment. As used herein, the term "nucleic acid segment", are smaller fragments of a nucleic acid, such as for non-limiting example, those that encode only part of the periaxin peptide or polypeptide sequence. Thus, a "nucleic acid segment" may comprise any part of the periaxin gene sequence(s), of from about 2 nucleotides to the full length of the periaxin peptide or polypeptide encoding region. In certain embodiments, the "nucleic acid segment" encompasses the full length periaxin gene(s) sequence. In particular embodiments, the nucleic acid comprises any part of the SEQ ID NO:1 and/or one of SEQ ID NO:27 through SEQ ID NO:53 sequence(s), of from about 2 nucleotides to the full length of the sequence disclosed in SEQ ID NO:1 and/or one of SEQ ID NO:27 through SEQ ID NO:53.



[0165] Various nucleic acid segments may be designed based on a particular nucleic acid sequence, and may be of any length. In certain embodiments, the nucleic acid segment may be a probe or primer. As used herein, a "probe" is a relatively short nucleic acid sequence, such as an oligonucleotide, used to identify other nucleic acid sequences to which it hybridizes. As used herein, a "primer" is a relatively short nucleic acid sequence used as a starting molecule for polymerization to extend from, such as in polymerase chain reaction, which is a method well known in the art. A non-limiting example of this would be the creation of nucleic acid segments of various lengths and sequence composition for probes and primers based on the sequences disclosed in SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:53.

[0166] The nucleic acid(s) of the present invention, regardless of the length of the sequence itself, may be combined with other nucleic acid sequences, including but not limited to, promoters, enhancers, polyadenylation signals, restriction enzyme sites, multiple cloning sites, coding segments, and the like, to create one or more nucleic acid construct(s). The overall length may vary considerably between nucleic acid constructs. Thus, a nucleic acid segment of almost any length may be employed, with the total length preferably being limited by the ease of preparation or use in the intended recombinant nucleic acid protocol.

[0167] In a non-limiting example, one or more nucleic acid constructs may be prepared that include a contiguous stretch of nucleotides identical to or complementary to SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:53. A nucleic acid construct may be about 3, about 5, about 8, about 10 to about 14, or about 15, about 20, about 30, about 40, about 50, about 100, about 200, about 500, about 1,000, about 2,000, about 3,000, about 5,000, about 10,000, about 15,000, about 20,000, about 30,000, about 50,000, about 100,000, about 250,000, about 500,000, about 750,000, to about 1,000,000 nucleotides in length, as well as constructs of greater size, up to and including chromosomal sizes (including all intermediate lengths and intermediate ranges), given the advent of nucleic acids constructs such as a yeast artificial chromosome are known to those of ordinary skill in the art. It will be readily understood that "intermediate lengths" and "intermediate ranges", as used herein, means any length or range including or between the quoted values (*i.e.* all integers including and between such values).

[0168] In particular embodiments, the invention concerns one or more recombinant vector(s) comprising nucleic acid sequences that encode a periaxin protein, polypeptide or

peptide that includes within its amino acid sequence a contiguous amino acid sequence in accordance with, or essentially as set forth in, SEQ ID NO:1, corresponding to human periaxin. In other embodiments, the invention concerns recombinant vector(s) comprising nucleic acid sequences that encode a mouse periaxin protein, polypeptide or peptide that includes within its amino acid sequence a contiguous amino acid sequence in accordance with, or essentially as set forth in SEQ ID NO:29. In particular aspects, the recombinant vectors are DNA vectors.

**[0169]** The term "a sequence essentially as set forth in SEQ ID NO:2 means that the sequence substantially corresponds to a portion of SEQ ID NO:2 and has relatively few amino acids that are not identical to, or a biologically functional equivalent of, the amino acids of SEQ ID NO:2.

**[0170]** The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein. Accordingly, a sequence that has between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids that are identical or functionally equivalent to the amino acids of SEQ ID NO:2 or at least one of SEQ ID NO:55 through SEQ ID NO:63 will be a sequence that is "essentially as set forth in SEQ ID NO:2" or "a sequence essentially as set forth in at least one of SEQ ID NO:55 through SEQ ID NO:63", provided the biological activity of the protein, polypeptide or peptide is maintained.

**[0171]** In certain other embodiments, the invention concerns at least one recombinant vector that include within its sequence a nucleic acid sequence essentially as set forth in SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:54. In particular embodiments, the recombinant vector comprises DNA sequences that encode protein(s), polypeptide(s) or peptide(s) exhibiting periaxin activity.

**[0172]** The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine and serine, and also refers to codons that encode biologically equivalent amino acids. Information on codon usage in a variety of non-human organisms is known in the art (see for example, Bennetzen and Hall, 1982; Ikemura, 1981a, 1981b, 1982; Grantham *et al.*, 1980, 1981; Wada *et al.*, 1990; each of these references are incorporated herein by reference in their entirety). Thus, it is contemplated that codon usage may be optimized for other animals, as well as other organisms such as fungi, plants, prokaryotes, virus and the like, as well as organelles that

contain nucleic acids, such as mitochondria, chloroplasts and the like, based on the preferred codon usage as would be known to those of ordinary skill in the art.

[0173] It will also be understood that amino acid sequences or nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, or various combinations thereof, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein, polypeptide or peptide activity where expression of a proteinaceous composition is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' and/or 3' portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

[0174] Excepting intronic and flanking regions, and allowing for the degeneracy of the genetic code, nucleic acid sequences that have between about 70% and about 79%; or more preferably, between about 80% and about 89%; or even more particularly, between about 90% and about 99%; of nucleotides that are identical to the nucleotides of SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:55 will be nucleic acid sequences that are "essentially as set forth in SEQ ID NO:1 or at least one of SEQ ID NO:27 through SEQ ID NO:55".

[0175] It will also be understood that this invention is not limited to the particular nucleic acid or amino acid sequences of periaxin. Recombinant vectors and isolated nucleic acid segments may therefore variously include these coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, and they may encode larger polypeptides or peptides that nevertheless include such coding regions or may encode biologically functional equivalent proteins, polypeptide or peptides that have variant amino acids sequences.

[0176] The nucleic acids of the present invention encompass biologically functional equivalent periaxin proteins, polypeptides, or peptides. Such sequences may arise as a consequence of codon redundancy or functional equivalency that are known to occur naturally within nucleic acid sequences or the proteins, polypeptides or peptides thus encoded. Alternatively, functionally equivalent proteins, polypeptides or peptides may be created via the application of recombinant DNA technology, in which changes in the protein, polypeptide or peptide structure may be engineered, based on considerations of the properties

of the amino acids being exchanged. Changes designed by man may be introduced, for example, through the application of site-directed mutagenesis techniques as discussed herein below, *e.g.*, to introduce improvements or alterations to the antigenicity of the protein, polypeptide or peptide, or to test mutants in order to examine periaxin protein, polypeptide or peptide activity at the molecular level.

[0177] Fusion proteins, polypeptides or peptides may be prepared, *e.g.*, where the periaxin coding regions are aligned within the same expression unit with other proteins, polypeptides or peptides having desired functions. Non-limiting examples of such desired functions of expression sequences include purification or immunodetection purposes for the added expression sequences, *e.g.*, proteinaceous compositions that may be purified by affinity chromatography or the enzyme labeling of coding regions, respectively.

[0178] As used herein the term "sequence" encompasses both the terms "nucleic acid" and "proteinaceous" or "proteinaceous composition." As used herein, the term "proteinaceous composition" encompasses the terms "protein", "polypeptide" and "peptide." As used herein "artificial sequence" refers to a sequence of a nucleic acid not derived from sequence naturally occurring at a genetic locus, as well as the sequence of any proteins, polypeptides or peptides encoded by such a nucleic acid. A "synthetic sequence", refers to a nucleic acid or proteinaceous composition produced by chemical synthesis *in vitro*, rather than enzymatic production *in vitro* (*i.e.* an "enzymatically produced" sequence) or biological production *in vivo* (*i.e.* a "biologically produced" sequence).

#### **G. Pharmaceutical Compositions**

[0179] Aqueous compositions of the present invention comprise an effective amount of a chemical compound or pharmaceutically acceptable salts thereof or the periaxin protein, polypeptide, peptide, epitopic core region, inhibitor, and/or such like, dissolved and/or dispersed in a pharmaceutically acceptable carrier and/or aqueous medium. Aqueous compositions of gene therapy vectors expressing any of the foregoing are also contemplated.

[0180] The phrases "pharmaceutically and/or pharmacologically acceptable" refer to molecular entities and/or compositions that do not produce an adverse, allergic and/or other untoward reaction when administered to an animal, such as a human, as appropriate.

[0181] As used herein, "pharmaceutically acceptable carrier" includes any and/or all solvents, dispersion media, coatings, antibacterial and/or antifungal agents, isotonic and/or absorption delaying agents and/or the like. The use of such media and/or agents for

pharmaceutical active substances is well known in the art. Except insofar as any conventional media and/or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. For human administration, preparations should meet sterility, pyrogenicity, general safety and/or purity standards as required by FDA Office of Biologics standards.

[0182] The biological material should be extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle, where appropriate. The active compounds may generally be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, intralesional, and/or even intraperitoneal routes. The preparation of an aqueous compositions that contain an effective amount of chemical compound or pharmaceutically acceptable salts thereof or a periaxin agent as an active component and/or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions and/or suspensions; solid forms suitable for using to prepare solutions and/or suspensions upon the addition of a liquid prior to injection can also be prepared; and/or the preparations can also be emulsified.

[0183] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions and/or dispersions; formulations including sesame oil, peanut oil and/or aqueous propylene glycol; and/or sterile powders for the extemporaneous preparation of sterile injectable solutions and/or dispersions. In all cases the form must be sterile and/or must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and/or storage and/or must be preserved against the contaminating action of microorganisms, such as bacteria and/or fungi.

[0184] Solutions of the active compounds as free base and/or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and/or in oils. Under ordinary conditions of storage and/or use, these preparations contain a preservative to prevent the growth of microorganisms.

[0185] A chemical compound or periaxin protein, polypeptide, peptide, agonist and/or antagonist of the present invention can be formulated into a composition in a neutral and/or

salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and/or which are formed with inorganic acids such as, for example, hydrochloric and/or phosphoric acids, and/or such organic acids as acetic, oxalic, tartaric, mandelic, and/or the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, and/or ferric hydroxides, and/or such organic bases as isopropylamine, trimethylamine, histidine, procaine and/or the like. In terms of using peptide therapeutics as active ingredients, the technology of U.S. Patents 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and/or 4,578,770, each incorporated herein by reference, may be used.

[0186] The carrier can also be a solvent and/or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and/or liquid polyethylene glycol, and/or the like), suitable mixtures thereof, and/or vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and/or antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and/or the like. In many cases, it will be preferable to include isotonic agents, for example, sugars and/or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and/or gelatin.

[0187] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and/or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, and/or highly, concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to the desired area.

[0188] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and/or in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and/or the like can also be employed.

[0189] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and/or the liquid diluent first rendered isotonic with sufficient saline and/or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and/or intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and/or either added to 1000 ml of hypodermoclysis fluid and/or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and/or 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0190] The chemical compound or pharmaceutically acceptable salts thereof or the active periaxin protein-derived peptides and/or agents may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, and/or about 0.001 to 0.1 milligrams, and/or about 0.1 to 1.0 and/or even about 10 milligrams per dose and/or so on. Multiple doses can also be administered.

[0191] In addition to the compounds formulated for parenteral administration, such as intravenous and/or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets and/or other solids for oral administration; liposomal formulations; time release capsules; and/or any other form currently used, including cremes.

[0192] One may also use nasal solutions and/or sprays, aerosols and/or inhalants in the present invention. Nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops and/or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, the aqueous nasal solutions usually are isotonic and/or slightly buffered to maintain a pH of 5.5 to 6.5. In addition, antimicrobial preservatives, similar to those used in

ophthalmic preparations, and/or appropriate drug stabilizers, if required, may be included in the formulation.

**[0193]** Additional formulations which are suitable for other modes of administration include vaginal suppositories and/or pessaries. A rectal pessary and/or suppository may also be used. Suppositories are solid dosage forms of various weights and/or shapes, usually medicated, for insertion into the rectum, vagina and/or the urethra. After insertion, suppositories soften, melt and/or dissolve in the cavity fluids. In general, for suppositories, traditional binders and/or carriers may include, for example, polyalkylene glycols and/or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

**[0194]** Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and/or the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations and/or powders. In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent and/or assimilable edible carrier, and/or they may be enclosed in hard and/or soft shell gelatin capsule, and/or they may be compressed into tablets, and/or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and/or used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and/or the like. Such compositions and/or preparations should contain at least 0.1% of active compound. The percentage of the compositions and/or preparations may, of course, be varied and/or may conveniently be between about 2 to about 75% of the weight of the unit, and/or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

**[0195]** The tablets, troches, pills, capsules and/or the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, and/or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and/or the like; a lubricant, such as magnesium stearate; and/or a sweetening agent, such as sucrose, lactose and/or saccharin may be added and/or a flavoring agent, such as peppermint, oil of wintergreen, and/or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials



may be present as coatings and/or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, and/or capsules may be coated with shellac, sugar and/or both. A syrup or elixir may contain the active compounds sucrose as a sweetening agent methyl and/or propylparabens as preservatives, a dye and/or flavoring, such as cherry and/or orange flavor.

#### **H. Lipid Formulations and/or Nanocapsules**

[0196] In certain embodiments, the use of lipid formulations and/or nanocapsules is contemplated for the introduction of a chemical compound or pharmaceutically acceptable salts thereof or periaxin protein, polypeptides, peptides and/or agents, and/or gene therapy vectors, including both wild-type and/or antisense vectors, into host cells.

[0197] Nanocapsules can generally entrap compounds in a stable and/or reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1  $\mu\text{m}$ ) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and/or such particles may be easily made.

[0198] In a preferred embodiment of the invention, the pharmaceutical may be associated with a lipid. The pharmaceutical associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. The lipid or lipid/pharmaceutical-associated compositions of the present invention are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape.

[0199] Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which are well known to those of skill in the art which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0200] Phospholipids may be used for preparing the liposomes according to the present invention and may carry a net positive, negative, or neutral charge. Diacetyl phosphate can be employed to confer a negative charge on the liposomes, and stearylamine can be used to confer a positive charge on the liposomes. The liposomes can be made of one or more phospholipids.

[0201] A neutrally charged lipid can comprise a lipid with no charge, a substantially uncharged lipid, or a lipid mixture with equal number of positive and negative charges. Suitable phospholipids include phosphatidyl cholines and others that are well known to those of skill in the art.

[0202] Lipids suitable for use according to the present invention can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma Chemical Co., diacetyl phosphate ("DCP") is obtained from K & K Laboratories (Plainview, NY); cholesterol ("Chol") is obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Preferably, chloroform is used as the only solvent since it is more readily evaporated than methanol.

[0203] Phospholipids from natural sources, such as egg or soybean phosphatidylcholine, brain phosphatidic acid, brain or plant phosphatidylinositol, heart cardiolipin and plant or bacterial phosphatidylethanolamine are preferably not used as the primary phosphatide, *i.e.*, constituting 50% or more of the total phosphatide composition, because of the instability and leakiness of the resulting liposomes.

[0204] "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes may be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). However, the present invention also encompasses compositions that have different structures in solution than the normal vesicular structure.

For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

[0205] Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and/or the presence of divalent cations. Liposomes can show low permeability to ionic and/or polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and/or results in an increase in permeability to ions, sugars and/or drugs.

[0206] Liposomes interact with cells *via* four different mechanisms: Endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and/or neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic and/or electrostatic forces, and/or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and/or by transfer of liposomal lipids to cellular and/or subcellular membranes, and/or *vice versa*, without any association of the liposome contents. Varying the liposome formulation can alter which mechanism is operative, although more than one may operate at the same time.

[0207] Liposome-mediated oligonucleotide delivery and expression of foreign DNA *in vitro* has been very successful. Wong *et al.* (1980) demonstrated the feasibility of liposome-mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa and hepatoma cells. Nicolau *et al.* (1987) accomplished successful liposome-mediated gene transfer in rats after intravenous injection.

[0208] In certain embodiments of the invention, the lipid may be associated with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda *et al.*, 1989). In other embodiments, the lipid may be complexed or employed in conjunction with nuclear non-histone chromosomal proteins (HMG-1) (Kato *et al.*, 1991). In yet further embodiments, the lipid may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression vectors have been successfully employed in transfer and expression of an

oligonucleotide *in vitro* and *in vivo*, then they are applicable for the present invention. Where a bacterial promoter is employed in the DNA construct, it also will be desirable to include within the liposome an appropriate bacterial polymerase.

[0209] Liposomes used according to the present invention can be made by different methods. The size of the liposomes varies depending on the method of synthesis. A liposome suspended in an aqueous solution is generally in the shape of a spherical vesicle, having one or more concentric layers of lipid bilayer molecules. Each layer consists of a parallel array of molecules represented by the formula XY, wherein X is a hydrophilic moiety and Y is a hydrophobic moiety. In aqueous suspension, the concentric layers are arranged such that the hydrophilic moieties tend to remain in contact with an aqueous phase and the hydrophobic regions tend to self-associate. For example, when aqueous phases are present both within and without the liposome, the lipid molecules may form a bilayer, known as a lamella, of the arrangement XY-YX. Aggregates of lipids may form when the hydrophilic and hydrophobic parts of more than one lipid molecule become associated with each other. The size and shape of these aggregates will depend upon many different variables, such as the nature of the solvent and the presence of other compounds in the solution.

[0210] Liposomes within the scope of the present invention can be prepared in accordance with known laboratory techniques. In one preferred embodiment, liposomes are prepared by mixing liposomal lipids, in a solvent in a container, *e.g.*, a glass, pear-shaped flask. The container should have a volume ten-times greater than the volume of the expected suspension of liposomes. Using a rotary evaporator, the solvent is removed at approximately 40°C under negative pressure. The solvent normally is removed within about 5 min. to 2 hours, depending on the desired volume of the liposomes. The composition can be dried further in a desiccator under vacuum. The dried lipids generally are discarded after about 1 week because of a tendency to deteriorate with time.

[0211] Dried lipids can be hydrated at approximately 25-50 mM phospholipid in sterile, pyrogen-free water by shaking until all the lipid film is resuspended. The aqueous liposomes can be then separated into aliquots, each placed in a vial, lyophilized and sealed under vacuum.

[0212] In the alternative, liposomes can be prepared in accordance with other known laboratory procedures: the method of Bangham *et al.* (1965), the contents of which are incorporated herein by reference; the method of Gregoriadis, as described in *DRUG*

*CARRIERS IN BIOLOGY AND MEDICINE*, G. Gregoriadis ed. (1979) pp. 287-341, the contents of which are incorporated herein by reference; the method of Deamer and Uster (1983), the contents of which are incorporated by reference; and the reverse-phase evaporation method as described by Szoka and Papahadjopoulos (1978). The aforementioned methods differ in their respective abilities to entrap aqueous material and their respective aqueous space-to-lipid ratios.

[0213] The dried lipids or lyophilized liposomes prepared as described above may be dehydrated and reconstituted in a solution of inhibitory peptide and diluted to an appropriate concentration with a suitable solvent, *e.g.*, DPBS. The mixture is then vigorously shaken in a vortex mixer. Unencapsulated nucleic acid is removed by centrifugation at  $29,000 \times g$  and the liposomal pellets washed. The washed liposomes are resuspended at an appropriate total phospholipid concentration, *e.g.*, about 50-200 mM. The amount of nucleic acid encapsulated can be determined in accordance with standard methods. After determination of the amount of nucleic acid encapsulated in the liposome preparation, the liposomes may be diluted to appropriate concentrations and stored at 4°C until use.

[0214] A pharmaceutical composition comprising the liposomes will usually include a sterile, pharmaceutically acceptable carrier or diluent, such as water or saline solution.

## **I. Kits**

[0215] Therapeutic kits of the present invention are kits comprising a chemical compound or pharmaceutically acceptable salts thereof or a periaxin protein, polypeptide, peptide, inhibitor, gene, vector and/or other periaxin effector. Such kits will generally contain, in suitable container means, a pharmaceutically acceptable formulation of a chemical compound or pharmaceutically acceptable salts thereof or a periaxin protein, polypeptide, peptide, domain, inhibitor, and/or a gene and/or vector expressing any of the foregoing in a pharmaceutically acceptable formulation. The kit may have a single container means, and/or it may have distinct container means for each compound.

[0216] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The chemical compound or pharmaceutically acceptable salts thereof or periaxin compositions may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, and/or other such like apparatus,

from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit.

[0217] However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

[0218] The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the chemical compound or pharmaceutically acceptable salts thereof or periaxin protein, gene and/or inhibitory formulation are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

[0219] The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, *e.g.*, injection and/or blow-molded plastic containers into which the desired vials are retained.

[0220] Irrespective of the number and/or type of containers, the kits of the invention may also comprise, and/or be packaged with, an instrument for assisting with the injection/administration and/or placement of the ultimate chemical compound or pharmaceutically acceptable salts thereof or a periaxin protein and/or gene composition within the body of an animal. Such an instrument may be a syringe, pipette, forceps, and/or any such medically approved delivery vehicle.

## **J. Methods of Making Transgenic Mice**

[0221] A particular embodiment of the present invention provides transgenic animals that contain the transgenic constructs of interest. In a specific embodiment there is a transgenic non-human animal whose genome comprises a transgene encoding a periaxin amino acid sequence, wherein said transgene is under the control of an operably linked promoter active in eukaryotic cells. In another specific embodiment the promoter is constitutive, tissue-specific, and/or inducible. In an additional specific embodiment, the animal is a mouse.

[0222] In a general aspect, a transgenic animal is produced by the integration of a given transgene into the genome in a manner that permits the expression of the transgene. Methods for producing transgenic animals are generally described by Wagner and Hoppe

(U.S. Patent 4,873,191; which is incorporated herein by reference), Brinster et al. 1985; which is incorporated herein by reference in its entirety) and in "Manipulating the Mouse Embryo; A Laboratory Manual" 2nd edition (eds., Hogan, Beddington, Costantini and Long, Cold Spring Harbor Laboratory Press, 1994; which is incorporated herein by reference in its entirety).

[0223] Typically, a gene flanked by genomic sequences is transferred by microinjection into a fertilized egg. The microinjected eggs are implanted into a host female, and the progeny are screened for the expression of the transgene. Transgenic animals may be produced from the fertilized eggs from a number of animals including, but not limited to reptiles, amphibians, birds, mammals, and fish.

[0224] DNA clones for microinjection can be prepared by any means known in the art. For example, DNA clones for microinjection can be cleaved with enzymes appropriate for removing the bacterial plasmid sequences, and the DNA fragments electrophoresed on 1% agarose gels in TBE buffer, with standard techniques. The DNA bands are visualized by staining with ethidium bromide, and the band containing the expression sequences is excised. The excised band is then placed in dialysis bags containing 0.3 M sodium acetate, pH 7.0. DNA is electroeluted into the dialysis bags, extracted with a 1:1 phenol:chloroform solution and precipitated by two volumes of ethanol. The DNA is redissolved in 1 ml of low salt buffer (0.2 M NaCl, 20 mM Tris, pH 7.4, and 1 mM EDTA) and purified on an Elutip-D™ column. The column is first primed with 3 ml of high salt buffer (1 M NaCl, 20 mM Tris, pH 7.4, and 1 mM EDTA) followed by washing with 5 ml of low salt buffer. The DNA solutions are passed through the column three times to bind DNA to the column matrix. After one wash with 3 ml of low salt buffer, the DNA is eluted with 0.4 ml high salt buffer and precipitated by two volumes of ethanol. DNA concentrations are measured by absorption at 260 nm in a UV spectrophotometer. For microinjection, DNA concentrations are adjusted to 3 mg/ml in 5 mM Tris, pH 7.4 and 0.1 mM EDTA.

[0225] Other methods for purification of DNA for microinjection are described in Hogan et al. Manipulating the Mouse Embryo (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986), in Palmiter *et al.* Nature 300:611 (1982); in The Qiagenologist, Application Protocols, 3rd edition, published by Qiagen, Inc., Chatsworth, CA.; and in Sambrook et al. Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989).

[0226] In an exemplary microinjection procedure, female mice six weeks of age are induced to superovulate with a 5 IU injection (0.1 cc, ip) of pregnant mare serum gonadotropin (PMSG; Sigma) followed 48 hours later by a 5 IU injection (0.1 cc, ip) of human chorionic gonadotropin (hCG; Sigma). Females are placed with males immediately after hCG injection. Twenty-one hours after hCG injection, the mated females are sacrificed by CO<sub>2</sub> asphyxiation or cervical dislocation and embryos are recovered from excised oviducts and placed in Dulbecco's phosphate buffered saline with 0.5% bovine serum albumin (BSA; Sigma). Surrounding cumulus cells are removed with hyaluronidase (1 mg/ml). Pronuclear embryos are then washed and placed in Earle's balanced salt solution containing 0.5 % BSA (EBSS) in a 37.5°C incubator with a humidified atmosphere at 5% CO<sub>2</sub>, 95% air until the time of injection. Embryos can be implanted at the two-cell stage.

[0227] Randomly cycling adult female mice are paired with vasectomized males. C57BL/6 or Swiss mice or other comparable strains can be used for this purpose. Recipient females are mated at the same time as donor females. At the time of embryo transfer, the recipient females are anesthetized with an intraperitoneal injection of 0.015 ml of 2.5 % avertin per gram of body weight. The oviducts are exposed by a single midline dorsal incision. An incision is then made through the body wall directly over the oviduct. The ovarian bursa is then torn with watchmakers forceps. Embryos to be transferred are placed in DPBS (Dulbecco's phosphate buffered saline) and in the tip of a transfer pipet (about 10 to 12 embryos). The pipet tip is inserted into the infundibulum and the embryos transferred. After the transfer, the incision is closed by two sutures.

#### **K. Gene Therapy Administration**

[0228] Where appropriate, gene therapy vectors can be formulated into preparations in solid, semisolid, liquid, or gaseous forms in the ways known in the art for their respective route of administration. Means known in the art can be utilized to prevent release and absorption of the composition until it reaches the target organ or to ensure timed-release of the composition. A pharmaceutically acceptable form should be employed which does not ineffectuate the compositions of the present invention. In pharmaceutical dosage forms, the compositions can be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds.

[0229] Accordingly, the pharmaceutical composition of the present invention may be delivered via various routes and to various sites in an animal body to achieve a particular



effect (see, *e.g.*, Rosenfeld *et al.* (1991); Rosenfeld *et al.*, (1991a); Jaffe *et al.*, 1992). One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Local or systemic delivery can be accomplished by administration comprising application or instillation of the formulation into body cavities, inhalation or insufflation of an aerosol, or by parenteral introduction, comprising intramuscular, intravenous, peritoneal, subcutaneous, intradermal, and topical administration.

[0230] One skilled in the art recognizes that different methods of delivery may be utilized to administer a vector into a cell. Examples include: (1) methods utilizing physical means, such as electroporation (electricity), a gene gun (physical force), or applying large volumes of a liquid (pressure); and (2) methods wherein said vector is complexed to another entity, such as a liposome or transporter molecule.

[0231] Accordingly, the present invention provides a method of transferring a therapeutic gene to a host, which comprises administering the vector of the present invention, preferably as part of a composition, with any of the aforementioned routes of administration or alternative routes known to those skilled in the art and appropriate for a particular application. Effective gene transfer of a vector to a host cell in accordance with the present invention can be monitored in terms of a therapeutic effect (*e.g.*, alleviation of some symptom or sign associated with the particular disease being treated) or, further, by evidence of the transferred gene or expression of the gene within the host (*e.g.*, with the polymerase chain reaction in conjunction with sequencing, Northern or Southern hybridizations, or transcription assays to detect the nucleic acid in host cells, or with immunoblot analysis, antibody-mediated detection, mRNA, or protein half-life studies, or particularized assays to detect protein or polypeptide encoded by the transferred nucleic acid, or impacted in level or function due to such transfer).

[0232] These methods described herein are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the compositions can be approximated further through analogy to compounds known to exert the desired effect.

[0233] Furthermore, the actual dose and schedule can vary depending on whether the compositions are administered in combination with other pharmaceutical compositions, or depending on interindividual differences in pharmacokinetics, drug disposition, and

metabolism. Similarly, amounts can vary in *in vitro* applications depending on the particular cell line utilized (*e.g.*, based on the number of vector receptors present on the cell surface, or the ability of the particular vector employed for gene transfer to replicate in that cell line). Furthermore, the amount of vector to be added per cell will likely vary with the length and stability of the therapeutic gene inserted in the vector, as well as the nature of the sequence, and is particularly a parameter which needs to be determined empirically, and can be altered due to factors not inherent to the methods of the present invention (for instance, the cost associated with synthesis). One skilled in the art can easily make any necessary adjustments in accordance with the exigencies of the particular situation.

[0234] The following examples are offered by way of example, and are not intended to limit the scope of the invention in any manner.

## EXAMPLE 1 MATERIALS AND METHODS

### Human subjects

[0235] All patients had DNA isolated from the peripheral blood, and lymphoblastoid cell lines were established.

### Human PRX cDNA sequence

[0236] The human *PRX* cDNA sequence corresponding to L-periaxin was defined by sequencing two EST clones (AW105547, AW337783) from the IMAGE consortium, by sequencing RT-PCR and 5' RACE products from human femoral nerve total RNA, and by sequencing 150-190 control chromosomes across all coding exons. Human femoral nerve total RNA was isolated using Trizol (Life Technologies) (Chomczynski and Sacchi, 1987). Prior to using the RNA for RT-PCR (One-Step RT-PCR or Superscript II RNase H Reverse Transcriptase, Life Technologies) or 5' RACE (GeneRacer Kit, Invitrogen), it was treated with ribonuclease-free deoxyribonuclease I (Life Technologies) to remove contaminating DNA. The products of the 5' RACE reaction were cloned into the TA vector (Invitrogen) to separate and sequence the various products.

### Mapping PRX

[0237] The published rat *Prx* cDNA sequence (GenBank Accession Number Z29649) was screened through the high-throughput genomic sequence database using the BLAST algorithm. BAC clone CTC-492K19 (AC010271) exhibited 83 percent identity to the cDNA

sequence. Using electronic PCR, nine chromosome 19q STSs in BAC CTC-492K19 were identified and these were used to place it on the chromosome 19 physical map. The RPCI-11 BAC library was also screened with an overgo primer probe for *PRX* and two BACs (104E13, 4K5) were isolated containing all coding exons of *PRX* and were used to map *PRX* by fluorescence *in situ* hybridization (FISH).

[0238] FISH was performed on metaphase preparations of human peripheral blood lymphocytes according to a modified procedure of Shaffer *et al.* (1997). Briefly, 200 ng of isolated BAC (104E13, 4K5) DNA was labeled by nick translation reaction using digoxigenin and 50 ng of chromosome 19q13.4 control cosmid probe (F13141 from LLNL flow sorted chromosome 19-specific cosmid library) using biotin (Boehringer Mannheim). Biotin was detected with FITC-avidin DCS (Vector Labs) and digoxigenin was detected with rhodamine-anti-digoxigenin antibodies (Sigma). Chromosomes were counterstained with DAPI diluted in Vectashield antifade (Vector Labs). Cells were viewed under a Zeiss Axioskop fluorescence microscope equipped with an appropriate filter combination. Monochromatic images were captured and pseudocolored using MacProbe 4.2.2/Power Macintosh G4 system (Perceptive Scientific Instruments, Inc., League City, TX USA).

### **Mutation Screening**

[0239] By aligning the human genomic sequence from BAC clone CTC-492K19 with the rat *Prx* cDNA, all coding exons were identified; each exon was confirmed following characterization of the human cDNAs. Using the Primer v3 program, primers were designed to amplify exons and intronic splice junctions and then were used to screen amplified PCR products from patient genomic DNA for mutations using the WAVE DNA-fragment analysis system (Transgenomic). Briefly, by PCR the coding region of *PRX* was amplified from 50 ng of patient genomic DNA using the primers listed in Table 1 and Qiagen HotStarTaq.

**TABLE 1**  
**PRIMER PAIRS USED FOR AMPLIFYING THE *PRX* CODING REGION AND**  
**OPTIMIZED DHPLC COLUMN TEMPERATURES FOR EACH AMPLICON**

Primer name	Primer pairs	DHPLC column temperature (°C)
Exon 4 F	GTAAGCATGGCCTCCACCT (SEQ ID NO:3)	63
R	CTCCTTGCTGCCCTAGTCTG (SEQ ID NO:4)	
Exon 5 F	ACCTGTTGAGCGCCAATG (SEQ ID NO:5)	66
R	CCCAAGGCAGATTCCTAACC (SEQ ID NO:6)	
Exon 6 F	CGTGCAAGTGGGCAGAACTA (SEQ ID NO:7)	65
R	TGACAAGACAGAGGGCAAGG (SEQ ID NO:8)	
Exon 7a F	AATACCAGGTGGGGCTCTTC (SEQ ID NO:9)	63
R	CTCTAGGCAGGGAAGTGTGG (SEQ ID NO:10)	
Exon 7b F	AGCCGTGGGAATCCAGGT (SEQ ID NO:11)	63
R	TGACACTTTGGGCAGCTCTA (SEQ ID NO:12)	
Exon 7c F	CAGAGGTTCTGACTCCCAGAG (SEQ ID NO:13)	62
R	GCCATCTCAGGCATTTTAGG (SEQ ID NO:14)	
Exon 7d F	CTGAGGTGAAACTCCCGAAG (SEQ ID NO:15)	63
R	GCAGAGTGAGAGAGGGGACA (SEQ ID NO:16)	
Exon 7e F	AAGCTAGGGAGGGCAGAGTC (SEQ ID NO:17)	63
R	AACTTGGGGAGAGCAAACCT (SEQ ID NO:18)	
Exon 7f F	CCTCAGGCAAGGTAGAGGTG (SEQ ID NO:19)	63
R	GTCACGGTGGGCATCTTAAA (SEQ ID NO:20)	
Exon 7g F	CAGGCTACAGGGTTCAGGTG (SEQ ID NO:21)	65
R	TTCTCTCTGACGGGGGACTT (SEQ ID NO:22)	
Exon 7h F	GTCCGCTTGCCACGTG TAG (SEQ ID NO:23)	62
R	GTACAGGCACTCCTGCCAGA (SEQ ID NO:24)	
S-PRX C F	CCGAGCCTTACAAAGTCTCCT (SEQ ID NO:25)	ND
S-PRX C R	AGTTTGGGGCAGAGAGGAAG (SEQ ID NO:26)	

ND: not determined

[0240] All forward primers had a -21 M13 primer tail (TGTAACGACGGCCAGT) and all reverse primers a M13 reverse tail (CAGGAAACAGCTATGACC). Each PCR product was generated, except that corresponding to exon 5, with the following conditions: 15 minutes at 95°C, 40 cycles of amplification (95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 1 minute), and 7 minutes at 72°C. For exon 5, 1.5 U of Qiagen HotStarTaq was added following the above protocol and then an additional 15 cycles of amplification was performed. To prepare the PCR products for DHPLC analysis, the products were pooled from every two patients, denatured for 5 minutes at 95°C, and reannealed by decreasing the temperature from 95 to 20°C over a period of 50 minutes. These PCR products were analyzed for heteroduplexes by DHPLC using a linear acetonitrile gradient (flow rate of 0.9ml/min, 2% slope (buffer A, 0.1 M triethylammoniumacetate; buffer B 0.1M triethylammoniumacetate/25% acetonitrile), column temperatures (Table 1)); optimal column temperatures were determined empirically and potential heteroduplexes were identified by visual inspection of elution chromatograms.

[0241] Using the Qiagen 96-PCR purification kit (Qiagen), patient PCR products having an abnormal elution profile and appropriate PCR products from relatives and control chromosomes were purified and sequenced with dye-primer chemistry (Applied Biosystems) using an ABI377 automated sequencer (Applied Biosystems). The resulting sequences were aligned and mutations were evaluated with the Sequencer sequence alignment program (ACGT Codes). The *PRX* cDNA sequence was numbered beginning with the adenine of the presumed initiating methionine, mutations are described according to den Dunnen and Antonarakis (2000).

## EXAMPLE 2

### MAPPING AND CHARACTERIZATION OF *PRX*

[0242] The *PRX* gene was mapped in the human genome (see Example 1). The cDNA sequence was defined, and the gene structure was characterized. The tissue expression profile of *PRX* mRNA was subsequently evaluated by standards well known in the art. By FISH and electronic PCR (Schuler, 1997), the BAC containing *PRX* (BAC CTC-492K19) maps to chromosome 19q13.13-q13.2 between *D19S324* and *D19S223* (FIG. 1a). This was confirmed by metaphase FISH; co-hybridization with BAC RPCI-11 104E13 (red) and chromosome 19 control cosmid F13141 (green) assigned *PRX* to 19q13.13-q13.2 (arrow, ISCN 1995) (FIG. 1a). This places *PRX* within a recently mapped interval for an autosomal

recessive myelinopathy (Delague *et al.* 2000). Sequencing of RT-PCR and 5' RACE products from femoral nerve mRNA and available EST clones defined two *PRX* transcripts of 4853 and 5502 bp excluding the polyA tails. The shorter mRNA is transcribed from seven exons and the deduced coding sequence extends from exon 4 through exon 7 (FIG. 1b). The longer transcript arises by retention of intron six (FIGS. 1b and 1c; FIG 2); this introduces a stop codon and results in a truncated protein with an intron encoded carboxyl terminus of 21 amino acids. The large periaxin protein (L-PRX) is encoded by the shorter spliced mRNA and the smaller periaxin protein (S-PRX) by the longer mRNA retaining intron 6. Coding regions are shaded (C).

[0243] As observed in mice and rats, the amino acid sequence deduced from the shorter cDNA sequence contains a PDZ domain (amino acids 14 to 98), a highly basic domain (amino acids 118 to 194) that functions as a nuclear localization signal in mice, a repeat domain (amino acids 400 to 700), and an acidic domain (amino acids 1098 to 1235, FIGS. 1b and 2) (Dytrych *et al.*, 1998; Gillespie *et al.*, 1994; Sherman and Brophy, 2000). The amino acid sequence deduced from the longer cDNA sequence contains only the PDZ motif. Hybridization of several Clontech multi-tissue Northernblots with a probe from exon 7 revealed expression of a 5.1 kb *PRX* mRNA in all tissues examined; spinal cord mRNA, a tissue with many peripheral nerve roots, showed strongest hybridization of 5.1 and 5.6 kb bands (FIG. 1c). In contrast to the nearly equal expression of each mRNA in mice (Dytrych *et al.*, 1998), the 5.6 kb mRNA appears less abundant in humans. RT-PCR confirmed the peripheral nerve tissue predominant expression.

### EXAMPLE 3 PRX MUTATION ANALYSIS IN NEUROPATHY PATIENTS

[0244] Using denaturing high pressure liquid chromatography (DHPLC), each coding exon of *PRX* was screened for mutations in 168 peripheral neuropathy patients who had tested negative for mutations involving *PMP22*, *MPZ*, *GJB1*, *EGR2*, or *MTMR2*. The PCR amplicons that gave an abnormal DHPLC elution profile were sequenced by standard methods in the art. Patient 851 of family HOU297 is compound heterozygous for deletion 2787 C and transition 2857C>T. By conceptual translation, 2787 C causes a frameshift after amino acid S929 and terminates the protein at codon 957 (S929fsX957), while 2857C>T causes the nonsense mutation R953X (FIGS. 3 and 4). The 2787 C or 2857C>T defects were not observed in control chromosomes (FIG. 4). The patient 1461 in family HOU579 is

compound heterozygous for deletion 2289 T and a 1102C>T transition causing the nonsense mutation R368X; 2289 T results in a frameshift after amino acid V763 and terminates the protein at codon 774 (V763fsX774, FIG. 3). The unaffected parents and son of family HOU579 are each heterozygous carriers of a *PRX* mutant allele (FIG. 3). Families HOU418, HOU579 and HOU297 exhibit autosomal recessive inheritance. Black symbols indicate DSN. Patient 851 from family HOU297 is compound heterozygous for mutations S929fsX957 and R953X; her older normal son is heterozygous for R953X. Patient 1461 from family HOU579 is compound heterozygous for mutations V763fsX774 and R368X; her normal brother is heterozygous for V763fsX774. Patient 1136 from family HOU418 has the homozygous mutation S929fsX957; her two normal sisters and her son are heterozygous for this mutation.

[0245] The defects 2289 T or 1102C>T were not observed in control chromosomes (FIG. 4). Patient 1136 of family HOU418 was homozygous for deletion 2787 C, the same deletion observed in patient 851 of HOU297. The unaffected parents, sisters, and son of this patient are each heterozygous carriers of this deletion on one *PRX* allele (FIGS. 3 and 4); although unaware of consanguinity, both parents hailed from a small village in Vietnam.

[0246] Other *PRX* sequence variants identified in patients and controls are shown in Table 2. In specific embodiments, these represent benign polymorphic variants. In one specific embodiment the alleles identified in only one control chromosome represent rare polymorphisms, or in an alternative embodiment a recessive carrier state.

TABLE 2

Alterations occurring in North American control chromosomes or unaffected family members

Alteration		Frequency in control chromosomes
3775G>A	E1259K	0/190*
1216G>A	A406T	1/178
4075-4077	E1359	1/150
1483G>C	E495Q	2/184
3394A>G	R1132G	6/182
3248C>G	P1083R	24/182
2763A>G	I921M	37/190
2645C>T	A882V	45/190
306C>T	T102T	ND
1491C>G	P497P	ND
2655T>C	P885P	ND

\*Observed in an unaffected sibling; ND, not determined

#### EXAMPLE 4

#### PHENOTYPE OF PATIENTS WITH PRX LOSS-OF-FUNCTION MUTATIONS

[0247] The clinical features of peripheral neuropathy in patients with autosomal recessive *PRX* mutations are comparable to those observed in the 19q13 linked family and the homozygous knockout mice (Table 3) (Delague *et al.*, 2000; Gillespie *et al.*, 2000). In each patient, objective findings include markedly reduced nerve conduction velocities and onion bulb formations on neuropathology. Interestingly, these patients have a more severe sensory component than usually seen with typical DSN or CMT1.

TABLE 3 CLINICAL FEATURES OF PATIENTS WITH MYELINOPATHY SECONDARY TO *PRX* MUTATIONS

Family	HOU297	HOU579	HOU418	CMT4F	<i>Prx</i> <sup>-/-</sup>
Patient	851	1461	1136	19q13.1-q13.3	mice
Current age (years)	46	6	31		
Sex	F	F	F		
	<7	1.5	1	Early childhood	4-6 weeks
Age at onset (years)					
Inheritance pattern	AR	AR	AR	AR	AR
Motor involvement	Distal dominant, severe	Distal dominant, severe	Distal dominant, severe	Distal dominant, severe	Severe weakness



Sensory loss	Severe	Severe	Severe	Severe	Severe
Sensory ataxia	No	Yes	Yes	Yes	Unsteady gait
Dysesthesia	None	None	Yes	Yes	Yes
Foot deformity	Pes cavus	None	Pes cavus	Pes cavus, Pes equinovarus Undetectable	Not described
Motor nerve conduction velocity	3 m/sec	Undetectable	2.1 m/sec in median nerve NA		Severely delayed
Peripheral nerve histopathology	NA	Hypomyelina- tion, Dysmyelinati on, OBF		Severe loss of MF, OBF	Demyelination, thick and thin myelin sheaths, loss of MF, OBF

**[0248]** The table utilizes the following abbreviations: F-female; AR-autosomal recessive; NA-not available; MF-myelinated fibers; and OBF-onion bulb formation.

#### **EXAMPLE 5 TESTING INDIVIDUAL FOR MYELINOPATHY**

**[0249]** In an embodiment of the present invention, a myelinopathy is diagnosed by the methods and/or compositions of the present invention. In a specific embodiment, a sample containing nucleic acid is obtained from an individual. In a preferred embodiment, the nucleic acid is SEQ ID NO:1 or an RNA from SEQ ID NO:1. Examples of samples include blood, saliva, semen, urine, hair, feces, sweat, tears, cheek scrapings, body tissue, and the like. The nucleic acid is analyzed by standard molecular biology methods, such as sequencing, polymerase chain reaction, hybridization, electrophoresis, or a combination thereof. In a specific embodiment the myelinopathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy syndrome (RLS). In the embodiment wherein polymerase chain reaction is used to diagnose the myelinopathy, primers selected from SEQ ID NO:3 through SEQ ID NO:26 may be utilized. In a specific embodiment the nucleic acid comprises an alteration such as 3775G>A, 1216G>A, 4075-4077d, 1483G>C, 3394A>G, 3248C>G, 2763A>G, 2645C>T, 306C>T, 1491C>G, and/or 2655T>C.

**[0250]** In a specific embodiment, a sample containing an amino acid sequence is obtained from an individual. In a preferred embodiment, the nucleic acid is SEQ ID NO:2.

Examples of samples include blood, saliva, semen, urine, hair, feces, sweat, tears, cheek scrapings, body tissue, and the like. The amino acid sequence is analyzed by standard molecular biology methods, such as with antibodies, electrophoresis, sequencing, or a combination thereof. In a specific embodiment the amino acid sequence comprises an alteration in SEQ ID NO:2 and may include E1259K, A406T, E1359del $\Delta$ , E495Q, R1132G, P1083R, I921M, A882V, T102T, P497P, and/or P885P. In a specific embodiment the myelinopathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy syndrome (RLS).

#### **EXAMPLE 6**

#### **IDENTIFYING COMPOUNDS FOR THERAPEUTIC USE**

**[0251]** In a specific embodiment there is a method of identifying a compound for the treatment of myelinopathy comprising the steps of exposing said compound to a knockout animal, wherein the animal comprises at least one defective allele of a nucleic acid sequence of SEQ ID NO:1 and wherein the animal has at least one symptom associated with the myelinopathy; and assaying for an improvement in the at least one symptom of the myelinopathy after exposure to said compound. In a specific embodiment there myelinopathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy syndrome (RLS). The compound may be any biological agent, such as a protein, lipid, nucleic acid, chemical agent, and the like. In a preferred embodiment, the knockout animal comprises two defective alleles of a nucleic acid sequence of SEQ ID NO:1.

**[0252]** A method of screening for a compound for the treatment of myelinopathy comprising the steps of providing a cell lacking a functional periaxin amino acid sequence contacting the cell with the compound; and determining the effect of the compound on said cell, wherein the effect on the cell is indicative of the treatment of the myelinopathy. In a specific embodiment there myelinopathy is Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), or Roussy-Levy syndrome (RLS). The compound may be any biological agent, such as a protein, lipid, nucleic acid, chemical agent, and the like.

**[0253]** In a specific embodiment there is a method of identifying an upregulator of periaxin nucleic acid sequence expression comprising the steps of administering a test compound to a transgenic animal, wherein the genome of said transgenic animal comprises a reporter nucleic acid sequence, wherein the sequence is under the control of an operably linked periaxin promoter active in eukaryotic cells; measuring the level of the periaxin expression; and comparing the level of the periaxin expression in the animal with normal periaxin expression, wherein an increase in the level following administration of the test compound indicates the test compound is an upregulator. The compound may be any biological agent, such as a protein, lipid, nucleic acid, chemical agent, and the like.

**[0254]** In another embodiment there is a method of identifying a drug having activity in the treatment of myelinopathy, comprising the steps of obtaining a compound suspected of having extracellular signaling activity; and determining whether the compound has the extracellular signaling activity. The extracellular signaling activity preferably is associated with the cytoskeleton. In a specific embodiment the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS). The compound may be any biological agent, such as a protein, lipid, nucleic acid, chemical agent, and the like.

#### **EXAMPLE 7 TREATING MYELINOPATHY**

**[0255]** In an embodiment of the present invention there is a method of treating myelinopathy in an organism, comprising the step of administering to the organism a therapeutically effective amount of a periaxin nucleic acid sequence, wherein the nucleic acid sequence is administered by a vector. In a specific embodiment, the vector is selected from the group consisting of a plasmid, a viral vector, a lipid, a liposome, a polypeptide, or a combination thereof. In another specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

**[0256]** In another embodiment of the present invention there is a method of treating myelinopathy in an organism comprising the step of administering to the organism a

therapeutically effective amount of a periaxin amino acid sequence, wherein the amino acid sequence is administered with a physiologically acceptable carrier.

[0257] In an additional embodiment there is a method of treating myelinopathy in an organism comprising the step of administering to the organism a therapeutically effective amount of a periaxin amino acid sequence, wherein the amino acid sequence is administered with a physiologically acceptable carrier. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

[0258] In another embodiment there is a method of treating an animal for a myelinopathy comprising the steps of identifying a compound which interacts with an amino acid sequence of SEQ ID NO:2; and administering to the animal a therapeutically effective amount of the compound. In a specific embodiment, the myelinopathy is selected from the group consisting of Charcot-Marie-Tooth (CMT) syndrome, hereditary neuropathy with liability to pressure palsies (HNPP), Dejerine-Sottas syndrome (DSS), congenital hypomyelinating neuropathy (CHN), and Roussy-Levy Syndrome (RLS).

[0259] In another embodiment there is a method of treating a patient for a myelinopathy comprising the steps of preparing a compound obtained by methods described herein; and administering the compound with a physiologically acceptable carrier to the patient.

#### EXAMPLE 7 SIGNIFICANCE OF *PRX* DEFECTS

[0260] Consistent with the phenotypes of *Prx*<sup>-/-</sup> mice, the three families described in the preceding examples establish that putative loss-of-function mutations in *PRX* cause autosomal recessive DSN (FIG. 4). The nonsense and frameshift mutations delete the carboxyl portion of L-periaxin, including the acidic domain. In a specific embodiment, the acidic domains mediate protein-protein interactions. Loss of this domain, therefore, in a specific embodiment inhibits binding of L-periaxin to the cytoskeleton or in an alternative embodiment precludes L-periaxin from interacting with proteins essential for transmission of extracellular signals.

[0261] *PRX* mutations are a significant cause of apparently sporadic and autosomal recessive DSN. Three of twenty unrelated DSN patients inherited two recessive mutant *PRX*

alleles; by comparison, four, three and two DSN patients of the twenty had *de novo* heterozygous causative mutations in *MPZ*, *PMP22* and *EGR2*, respectively. Moreover, because HOU297, HOU579, and HOU418 are respectively of North American Hispanic, Northern European (English-German-Polish), and Vietnamese ethnicities, in a specific embodiment *PRX* mutations are a significant cause of DSN in most populations. Thus, identification of *PRX* mutations is important for the diagnosis and recurrence risk counseling of DSN patients and their families.

[0262] Mutations of the transcription factor *EGR2* cause myelinopathies (Warner *et al.* 1998), and in a specific embodiment mutation of genes regulated by *EGR2* also result in myelinopathies. In a specific embodiment, the expression of proteins interacting with L-periaxin is also regulated by *EGR2*.

[0263] The association of mutations in *PRX* with peripheral neuropathy not only identifies another genetic cause for the CMT1 spectrum of myelinopathies but also provides further insights into the molecular mechanisms for these diseases. The interaction among L-periaxin, the cytoskeleton and a membrane complex is reminiscent of the interactions among the proteins of the dystrophin-sarcoglycan complex (Cohen and Campbell, 2000) and the signaling complexes organized by other PDZ domain proteins (Montell, 2000). In a specific embodiment, mutations in cytoskeletal and membrane proteins interacting with L-periaxin also cause CMT or related neuropathies.

## EXAMPLE 8

### *PRX* MUTATIONS CAUSE A BROAD SPECTRUM OF DEMYELINATING NEUROPATHIES

#### *PRX* mutation analysis

[0264] By DNA sequencing, each coding exon of *PRX* was screened for mutations in 29 peripheral neuropathy patients who had tested negative for mutations involving the following genes: *PMP22* encoding peripheral myelin protein, *MPZ* encoding myelin protein zero, *GJB1* encoding connexin 32, and *EGR2* encoding early growth response 2 protein. Sibling patients PN-44.1 and PN-44.4 were homozygous for the mutation 2145T>A that by conceptual translation causes a nonsense stop codon at amino acid 715 that normally encodes a cysteine (C715X). Patient PN-761.3 was homozygous for 247DC that results in frameshift mutation R82fsX96. The unaffected parents, sister and brothers either did not carry the

mutation or were heterozygous carriers (FIG. 5). We did not observe either 2145T>A or 247DC in 180 control chromosomes.

### **Histopathology**

[0265] For patient PN-44.1, light microscopy revealed a severe loss of myelinated axons of all diameters and increased connective tissue (FIG. 6A). Although some remaining myelinated fibers were normal, many showed tomacula formation or small onion bulb formations. Some onion bulbs had a central axon but many were denervated. There was no evidence of axonal regeneration. Endoneural and perineurial vessels were normal and inflammatory infiltrates were not seen. On electron microscopy, the tomacula consisted of concentric or eccentric thickenings of the myelin sheath with focally folded myelin surrounding a constricted axon. The onion bulbs were made up of concentrically arranged Schwann cell processes with or without a central axon. Multiple paranodal abnormalities were identified including a reduced number of myelin loops and an absence of septate-like junctions between the paranodal myelin and the axon (FIG. 7B). Denervated Schwann cell units of unmyelinated axons enclosed collagen pockets.

[0266] For patient PN-761.3, light microscopy revealed a severe loss of thick myelin sheaths. Numerous fibers were demyelinated or thinly remyelinated. Atrophic axons with relatively thick myelin sheaths (tomacula) were occasionally seen (FIG. 6B). Typical onion bulb formation was minimal. At the electron microscopic level, basal lamina onion bulbs with up to 6 layers were frequently encountered. As observed in patient PN-44.1, the paranodes of nerve fibers showed incomplete myelination or demyelination and separation of multiple terminal myelin loops from the axon at the paranode, sometimes with flat intervening Schwann cell processes (FIG. 7A). The unmyelinated axons were of uneven size and surrounded by Schwann cell processes that showed degenerative changes such as condensation, swelling, vacuoles, membranous cytoplasmic bodies, and dilated ergastoplasma.

### **Periaxin immunofluorescence analysis**

[0267] Immunofluorescence analysis of a normal human sural nerve biopsy showed positive double staining for myelin basic protein (MBP) and the anti-N-terminal, the anti-repeat region and the C-terminal L-periaxin antibodies. In patient 44.1, there is also staining for the N-terminal and repeat region antibodies, but there is no labeling with the anti-C-

terminal antibody even though there is MBP-positive staining (FIG. 8). This result demonstrates that a truncated L-periaxin is made. It also indicates that the C-terminal region downstream of the repeat region has an important function. Furthermore, these data suggest that interaction with DRP2 (approximately at amino acid positions 118 to 196 in rat L-periaxin) is not sufficient for L-periaxin's function.

[0268] These two families confirm that putative loss-of-function mutations in *PRX* cause autosomal recessive neuropathies and broaden the spectrum of *PRX*-associated peripheral neuropathies. Consistent with the phenotype of *Prx*<sup>-/-</sup> mice (Gillespie *et al.*, 2000) and previously reported patients (Boerkoel *et al.*, 2001; Gulbot *et al.*, 2001), all three patients reported in this study had marked sensory involvement. Such severe sensory involvement is rare among patients with mutations in other CMT-associated genes such as PMP22, MPZ, GJB1 and EGR2 and thus may be a signature clinical feature of neuropathy arising from *PRX* mutations. Interestingly, the sensory involvement observed in patients PN-44.1 and PN-44.4 was more severe, and their motor neuropathy was less severe, than previously reported for *PRX* mutations (Boerkoel *et al.*, 2001; Gulbot *et al.*, 2001).

[0269] Patient PN-761.3 has been the only patient reported with a *PRX* mutation affecting both L- and S-periaxin; all other patients have had mutations involving only L-periaxin. A thorough evaluation of her symptoms and nerve histopathology did not identify features that were distinct from those observed in other patients; therefore no specific pathology can be attributed to the frameshift mutation in S-periaxin. Because the frameshift mutation occurs in exon 6 (the penultimate exon of L-periaxin), in a specific embodiment this mutation results in complete loss of L-periaxin expression by nonsense mediated RNA decay (Lykke-Andersen, 2001). In contrast, because exon 6 is the last exon of S-periaxin, in a specific embodiment S-periaxin is expressed as an altered protein. Expression of this altered form of S-periaxin may therefore fulfill the function of S-periaxin.

[0270] The peripheral nerve pathology observed in the patients described herein include demyelination with minor remyelination, typical or basal lamina onion bulb formation with occasional tomacula, focally folded myelin, and detached terminal myelin loops. Focally folded myelin has been reported in patients with myotubularin related protein 2 (MTMR2) mutations (Houlden *et al.*, 2001), a patient with an MPZ mutation (Nakagawa *et al.*, 1999) and a patient with an EGR2 mutation (Timmerman *et al.*, 1999). However, detachment of terminal paranodal myelin loops from the axon with loss of septate-like

junctions and transverse bands has not yet been reported with these mutations. Therefore detached terminal myelin loops with focally folded myelin may be unique to the pathology observed with mutations of *PRX*. On the other hand, separation of terminal myelin loops from the axon by flat intervening Schwann cell processes was also seen in HNPP and Cockayne Syndrome (Schroder, 1996).

[0271] Recent data show that L-periaxin is an integral constituent of a dystroglycan-dystrophin-related protein 2 complex in the plasma membrane, where it presumably participates in interaction with the basal lamina surrounding the Schwann cell (Sherman *et al.*, 2001). The importance of this complex for stabilizing the axon-Schwann cell unit is illustrated by periaxin-null mice, which show a late-onset peripheral demyelinating neuropathy (Gillespie *et al.*, 2000) and the observation of CMT4F and Dejerine-Sottas disease among patients with *PRX* mutations (Boerkoel *et al.*, 2001; Guilbot *et al.*, 2001). A previously described patient with CMT4F and a homozygous mutation (R196X) did not express the periaxin protein (Guilbot *et al.*, 2001). However, as described herein there is a patient with a similar demyelinating neuropathy who expresses a truncated periaxin protein lacking the C-terminal region. The interactions of this domain of the periaxin protein would seem to be critical in maintaining peripheral nerve myelination. This C-terminal domain is responsible for targeting the protein for ubiquitin-mediated proteolysis. Hence, the presence of this truncated protein in a specific embodiment reflects an enhancement of its stability. Therefore, it cannot be ruled out that, in addition to a loss-of-function caused by the absence of the C-terminal domain, this mutant protein may also disrupt interactions with the basal lamina as a result of gain-of-function effects.

[0272] Although the interaction between periaxin and DRP2 may be essential for complex formation, the disruption of the interaction between these two proteins does not appear to be essential for causation of demyelinating CMT. Immunofluorescence studies on the nerve biopsy from patient PN-44.1 showed that this patient made a stable truncated periaxin protein containing the DRP2 binding domain, and this suggests that the truncated protein can still interact with DRP2. In addition, 168 patients with CMT and related neuropathies were screened for DRP2 mutations but did not identify any nucleotide sequence variants segregating with disease. Thus these two observations suggest that mutation of DRP2 is not a prominent cause of demyelinating CMT neuropathy.



[0273] Similar to the spectrum of phenotypes observed with mutation of other genes associated with CMT and related inherited peripheral neuropathies, the clinical phenotypes manifested in patients with mutations in *PRX* include CMT myelinopathies and DSN. However, in contrast to mutation of other neuropathy genes, mutation of *PRX* causes a prominent sensory neuropathy. These observations on peripheral neuropathy due to recessive *PRX* mutations add to a growing body of evidence implicating specific genes/proteins in peripheral nerve function and delineating the pathological consequences of their dysfunction.

[0274] FIG. 9 provides a summary of all reported mutations identified in *PRX*. Materials and methods for this Example are as follows.

### **Human subjects**

[0275] DNA was isolated from the peripheral blood of each patient.

### **Mutation Screening**

[0276] Mutation screening was performed as described (Boerkoel *et al.*, 2001) The *PRX* cDNA sequence was numbered beginning with the adenine of the presumed initiating methionine and described mutations according to den Dunnen and Antonarakis (den Dunnen and Antonarakis, 2000).

### **Sural nerve pathology**

[0277] Sural nerve biopsies, performed at the age of 40 years in patient PN-44.1 and at the age of 3 years in patient PN-761.3 were analyzed according to standard morphological procedures for light- and electron microscopy (Delague *et al.*, 2000; Schroder, 1996). No morphometric studies were done.

### **Immunohistochemistry**

[0278] Frozen sections (5  $\mu$ m) of sural nerve biopsy embedded in OCT (optimal cutting temperature) were collected on 3-aminopropyltriethoxysilane-subbed slides. The sections were fixed in 4% paraformaldehyde solution. Immunofluorescence for L-periaxin, myelin basic protein, and neurofilament was then carried out as described (Dytrych *et al.*, 1998).

### **Patients**

#### **Family PN-44**

[0279] The proband (PN-44.1, FIG. 4) was the sixth child of healthy consanguineous parents. One brother died at the age of 3 months due to heart failure, two sisters were

healthy, and one sister and one brother had a similar neurologic phenotype. The affected sister died at the age of 48 years due to a cardiomyopathy; detailed clinical information on her neurologic condition was not available. The affected brother (PN-44.4) is described below.

**[0280]** Beginning in the first year of life, patient PN-44.1 had signs of motor involvement with difficulty sitting and subsequently delayed acquisition of motor milestones and inability to run as fast as children of her own age; she attended a school for children with a motor handicap. She developed scoliosis in puberty. A neurological examination at the age of 50, showed a normal mental status, normal cranial nerves except for hearing loss, weakness of the intrinsic hand (5-/5), foot (5-/5) and distal leg muscles (5-/5), atrophy of the thenar and foot muscles, a steppage and ataxic gait, absent tendon reflexes, s-curved scoliosis, and pes cavus. Her strength was normal in more proximal muscles. Co-ordination tests showed slight dysmetria on the finger-nose test that could have been due to proprioceptive problems. She had severely decreased sensitivity for touch, position, vibration, pin-prick and temperature to the level of the knees and elbows. She had no palpable nerve hypertrophy. Electrophysiological studies of the median and ulnar nerves showed slow motor nerve conduction velocities (motor NCV, 3 m/sec), reduced compound muscle action potentials (CMAP, median: 1.1 mV, ulnar: 0.45 mV, control: > 6mV), and undetectable median sensory nerve action potentials.

**[0281]** Patient PN-44.4 had gait problems from childhood and developed a severe scoliosis at the age of 10 yrs. On the examination at the age of 54 years, he had normal mental status, normal cranial nerves excepting hearing loss, absent reflexes, weak foot and distal leg muscles, and atrophy of the hands, distal forearms and calves. His proximal muscle strength was normal. His sensation for touch, position, vibration, and pin-prick was severely reduced in both arms and legs. He had no palpable hypertrophic nerves and no pes cavus. The results of his electrophysiologic studies were unavailable.

#### **Family PN-761**

**[0282]** Patient PN-761.3 was the first child of healthy consanguineous parents (FIG. 4) who had normal motor nerve conduction velocities. Her three siblings were healthy. Beginning in the first year of life, she manifested delayed motor development; she sat at 10 months, crawled at 17 months, stood with support at 4 years and took her first steps at 5 years. At 6 years, she was able to walk 20-30 meters with a broad based gait and marked

sensory ataxia. On examination at 2 years of age, she had absent deep tendon reflexes, weakness of her lower legs and hands, atrophy of the distal lower leg muscles, incomplete foot dorsal flexion, pes planus, and normal cranial nerves. She could stand only with Nancy Hilton orthoses. Her proximal muscle strength and head control were good, and her spine was straight. When last seen at age 6 years, she had weak proximal muscles, could not rise from a squat, walked with 'locked knees', and was unable to hop on two legs or stand on one leg. She was able to stand briefly on her toes but not on her heels. Additionally, her sensation of vibration was diminished in the distal lower legs and hands. and her tongue showed fasciculations. Her electrophysiologic studies at age 20 months showed a normal sensory nerve conduction velocity (sensory NCV, 53 m/sec), latency (1.3 msec) and action potential (SNAP, 21mV), but undetectable CMAP upon stimulation of the peroneal and tibial nerves (100 mA for 0.5 sec). Her visual evoked potential was normal and her auditory evoked potential showed a slight delay of wave I.

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All patents and publications mentioned in the specification are indicative of the level of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

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U.S. Patent No. 5,935,791, issued Aug. 10, 1999  
U.S. Patent No. 5,935,825, issued Aug. 10, 1999  
U.S. Patent No. 5,939,291, issued Aug. 17, 1999  
U.S. Patent No. 5,942,391, issued Aug. 24, 1999

European Application No. 320 308

European Application No. 329 822

GB Application No. 2 202 328

PCT Application No. PCT/US87/00880

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One skilled in the art to which the invention pertains readily appreciates that the patent invention is well adapted to carry out the objectives and obtain the ends and advantages mentioned as well as those inherent therein. Periaxin, periaxin mutations, methods, techniques, pharmaceutical compositions, treatments, and procedures described herein are presently representative of the preferred embodiments and are intended to be exemplary and are not intended as limitations of the scope. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention or defined by the scope of the pending claims.